Science Fair Logbook

Logged by Isavella Tsoulias and Serena Lu

Dec 12 2024

- Chosen project topic: Phage therapy and bacteriophages
- Creating timeline
    - Have weekly meetings on Wednesdays and Fridays and maybe Saturdays
    - Rest of December:
        - Collecting background information and doing research on the different focuses in our topic
        - Brainstorm different experiments/testable questions to do
    - The first week of January:
        - Planning the project and how to perform the experiments
        - Have a definite idea and plan by the end of that week
    - Up to the first week of February
        - Completing the project/experiment
        - Doing the actual hands-on work
    - Rest of February till March
        - Creating trifold and our work to present at the science fair
- Different prompts to keep in mind when we are researching
    - Evolution of phage therapy from advantages to obstacles?
    - The different applications and research areas in phage therapy?
    - The innovation in the field?
    - What is it and how does it work?
- Today was planned our timeline and thought of ideas and things to research for science fair

Dec 18 2024

- Brainstormed different ideas/experiment possibilities to do
- Narrowed it down to two ideas:
    - Genetically engineering phages
    - Testing phage effectiveness in different environments (temperature, pH)

Dec 20 2024

- Reviewed the two ideas and have expectations set for next meeting
    - Each person is to create a list of materials and costs for the experiment
    - Also needs a rough procedure outline

Dec 22 2024

- Did research to see what we would need to do a real-life experiment of genetically engineering phages along with estimated costs and a rough procedure outline
- Looking at this it seems like it is not feasible to do in real life due to costs of the products and the restrictions on equipment, if we were to do this, which is very unlikely, we would probably need these at the least
    - https://www.thermofisher.com/ca/en/home/life-science/genome-editing/crispr-nuclease-vector.html That is what we could use to create our tailored gene editing system
    - https://norgenbiotek.com/product/phage-dna-isolation-kit This is what we could use to extract phage DNA
    - https://www.carolina.com/viruses/basic-bacteriophage-culture-set-a/121150.pr We could use set A or B depending on what we want to do

- This experiment is still 100% feasible with online simulations
- Procedure if we were to genetically engineer the phages online
    - Identify genes to target and design edits
        - Use tools like benchling to analyze the chosen genome, identify genes of interest, and design the gRNA for CRISPR-based editing
        - Identify genes responsible for whatever we want to be editing or improving on
        - Design the gRNA to make the edits
        - Use software like Benchling, NCBI BLAST (identify homologous regions or sequences of interest), MEGA (additional gene analysis)
    - Simulate the actual genome edits to create our newly gene edited phages
        - Import genome and define cut sites using our gRNA and then complete the insertion or replacement of the DNA sequences
        - Validate the edits
        - Visualize them for diagrams
        - Software used like SnapGene Viewer, Python libraries such as Biopython and CRISPResso2 (for in silico genome editing and analyzing the CRISPR edits), InDelphi (predict insertions and deletions for targeted editing)
    - Simulate and compare edited vs non-edited phages
        - Define parameters for the simulation
        - Write simulation script or use COPASI
        - Run multiple simulations to compare things such as killing efficiency, time to clear bacterial populations, potential off-target effects, etc.
        - Software such as Python, Octave (for mathematical modelling of population dynamics), COPASI (simulate biological pathways and infection dynamics)
    - Analyze results
    - Compare the metrics such as infection efficiency, host range specificity, overall population dynamics, etc. (based on the type of gene edits we make anyway)
    - Create visualizations such as growth curves, time-lapse comparisons, etc.
    - Use software such as R or Python (for statistical analysis and visualization), Google Sheets (simple plotting and comparisons)

The general stuff needed for the experiment(might be difficult to do)

Materials Needed

- Environmental samples: Water, soil, or other relevant samples
- Phage lysates : where to buy https://www.bio-world.com/cell-lysis-buffers/phage-lysis-buffer-p-30629559?srsltid=AfmBOori3im83RXFC0zS4tMkQ8N8FHsdSD0jFfg3qFARylvlLmOnuEvZ)

- Bacterial cultures : Susceptible to the phages we're studying TBD
- LB broth : hem grow the bacteria

(Recipe: https://med.wmich.edu/sites/default/files/Pioli_Lab_LB_Media_Recipe.pdf- can get most ingredients from home or school
Where to buy:

https://www.sigmaaldrich.com/CA/en/product/sigma/l3022?utm_source=google&utm_medium=organicshopping&gad_source=1&gclid=EAIaIQobChMIg7W588C-igMVCCWtBh0cEy1DEAQYASABEgIVw_D_BwE)

- Petri dishes and agar plates :store bacteria
- Incubator : For maintaining controlled environment .

(really pricey might make project hard to do)

- Microscope
- Plaque assay kits : For quantifying phage infectivity

(really pricey might make project hard to do)

- Pipettes and sterile tips
- Labels and markers : marking all samples and experimental setups
- Safety equipment : Gloves, lab coat, and eye protection

Procedure (kinda)

1. Collect environmental samples using sterile techniques and store them appropriately.
2. Process samples to isolate bacteria and phages. This may involve filtration, centrifugation, and other purification steps.
3. Grow bacterial cultures in LB broth to the desired density.
4. Add phage lysates to bacterial cultures at a known multiplicity of infection
5. Incubate the cultures under controlled conditions to allow phage infection and bacterial lysis.
6. Use plaque assays to quantify phage infectivity and determine the effectiveness of phages in different environments.
7. Analyze

Dec 29 2024

- Looked to see if we can create our own plaque assays
- The homemade plaque assays aren't feasible to yield accurate results or promote growth for our organisms

Jan 8 2025Created list and cost

- Phage lysates + Bacterial Culture: ~$52.85
  - https://www.carolina.com/viruses/basic-bacteriophage-culture-set-a/121150.pr
- Agar plates: ~$37.95
  - Nutrient Agar Plates: https://www.amazon.ca/Nutrient-Sterile-Petri-Dishes-Cotton/dp/B00B79BD3Q/ref=sr_1_5?crid=1UMSGD4DBEHR8&dib=eyJ2IjoiMSJ9.w08F8SaLqEsRQ5GwiPZBuWjmKvelyOp1q6KrHE4pHZ5H9K2z8rK0KQ_GN5FyoODWY6w6T-nF5bkG3TB2sWLNjoizYqmajRJBQOxdczr2uFhKuWlz59VqHMwjalTcUbYZZwgXiVIJbk6CNnzLJ2YXAtGmiGvW9SI_1yN5uRVi1uVfu4CI_VVwkSUbOb-ISdLGMCoLr3iEkkOlFusEEI-FqOS7ce2YrGnA3UPJeLClgowB36C2_k7vtxoK0lgfoV4UQOcy2-_1DoF7bz

WQ1tBTUgsSJ-kWpHCUDMmZiVlZKZqvD8rFM-yaMjbR7-EtbaHZP5m-BUHsH0Ogq5LRrkM1V-lS_vJQDIWlqLaaFPB8t7IcHhO0Syn4V-yfmvkoqa0qEhat2NfxeFqFB3OnII0chbgKJ7R5e-LE9O4b093r8XNFWDZaIfSilS1ezqAV9iSo._CmWjNvTXb6TK1k6BdvPJL-f9XXzhO3RQTurTZUbhb0&dib_tag=se&keywords=agar+plates&qid=1736484555&sprefix=agar+pl%2Caps%2C129&sr=8-5

- Agar agar powder for soft agar overlay: ~$15.99
  - https://www.amazon.ca/Agar-Powder-2oz-Excellent-Strength/dp/B008SV6OLM/ref=sr_1_13?crid=IT6P3XSFHIUV&dib=eyJ2IjoiMSJ9.PiYKxFpoRP0vX09mSfc0bcmpx4KswxRrDJ7ukgyvUtXLEifVYFz4QQKWmE9hl4Bw20XnitfFG9v8PYeOgkfacngvMU3is2g6MyHkxiO_VSv7QFOrRuZXrdYnEAVVc0MRHmogzYQNWrmSnticU3CR_W0wnKCWJEsFqYuz7P1kcjdpRdriuHuczLGxkRKwbxOLHhkcOBnmLQb3uteDgRBamQmjWxDSD-xFnsU9U1eUpTcJX8srjVbGTInLgoqfqZbCaR1Dehe5qB74x-iQCb7unz738onbz-blesHAM5qyaXmc6GKpvBecwd3W_7DABIVgrfojjM3OP4q2u0MOd4vDyq9JwLoYXxNk5zF52hPNjs5QEQTKxLGPnnqMVPS1LzXE1NnyUi5lj7rmNBfotuFWUWQRlo6lg6ck5YvcFTgmnujEVrsX8KtJZbF-BYFR5DWa.jX3qDlizrA955yrrdIfj5XFwxWCw6l3elDyshlt6xAs&dib_tag=se&keywords=soft+agar&qid=1736484913&sprefix=soft+alar%2Caps%2C164&sr=8-13
- Sterile water (needs to be heat treated): ~$1.92
  - https://www.walmart.ca/en/ip/Great-Value-4L-Distilled-Water/6000143710020
- LB Broth Powder (maybe free): ~$33.58
  - https://www.biobasic.com/lb-broth-lennox
- Inoculation loops (need to be heat treated): ~$9.50
  - https://www.amazon.ca/Inoculating-Bacterial-Microbiology-Re-Reusable-Inoculation/dp/B082L23VH3/ref=sr_1_2_sspa?crid=3OA1N9KQ0CVBR&dib=eyJ2IjoiMSJ9.EtiJIslZpuvC7YSkt_NU0dh37sJDsJXyDPHjI3_XTjHVFxtXQcLtw3UScqDsUfMvlK10MDvaS6s_GVhNi7l4gIJsYHx9dKde4WGWPTnL0S0b-e_bSrpfCreM7OoU7uZC3UmF2L0cP1z_4yNVn9JDXBooX7xWYTrHf56Mni0afcs1AwfTQyOHJwy3o1ozotlTg77iLTCWbjpU8l08ejkSQxoHG9CvvMyVqIbN3_EzoVz-Q5zQoLFjtr_lQAK4Tw3FbAmtzgl4NWa4bw1wCAL_Rv0NGrmtT_HH9P9H6GVFn4Y.D-OiTW2AwkyRXD9LfPbV0or9lTvsM9x5uupaEjIQdFE&dib_tag=se&keywords=sterile+inoculation+loop&qid=1736486816&sprefix=sterile+inoc%2Caps%2C134&sr=8-2-spons&sp_csd=d2lkZ2V0TmFtZT1zcF9hdGY&psc=1
- Pipettes
  - https://www.amazon.ca/CCTVMTST-Disposable-Graduated-Transfer-Pipettes/dp/B09J4M9TGT/ref=mp_s_a_1_7?adgrpid=60263225439&dib=eyJ2IjoiMSJ9.Gm3VT0s4U0fWB37Ob2wHYfS41XTNxNrNar2_h70-nwOKfQVPFNAjZBMEXFTGhGJeWi5ZxwlOkJd8caOVSvaX0stmAyS8OnPnkoBChcqLpBHbcTR80hPwQRdB3NNIS1D_3Yzq-jYsKCx6S4IcejEIIFOmZ3sngJaDbYypJ2X66LHIbiGxBQYc-a5lsIi_i0I1ABLqiOW3ZRQI_v4udZ73qg.6RogFgvzRdENcs4k8jYYEE7uKQ8I6bWQoPV4CptXq6M&dib_tag=se&hvadid=310035911899&hvdev=m&hvlocphy=9194528&hvnetw=g&hvqmt=e&hvrand=6150386584991984820&hvtargid=kw

d-21707296&hydadcr=11874_10122450&keywords=pipettes&qid=1736550591&sr=8-7

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Did Research(just wrote notes for this article :')
Influence of Environmental Factors on Phage–Bacteria Interaction and on the Efficacy and Infectivity of Phage P100

Background Info+Terminology

Bacteriophages (Phages) - Viruses that infect and kill specific bacteria
Used in food safety to control pathogens like Listeria monocytogenes
Advantages - High host specificity, no alteration of food properties
Listeria monocytogenes - Food-borne pathogens common in harsh environments (low pH, high salt, low temperatures) Appear Frequently in food production facilities
Phage P100 - Commercially used against L monocytogenes (ListexTM P100)
FDA-approved as GRAS (Generally Recognized as Safe)
Centrifuge - a device that helps separate components of a mixture through the use of centrifugal force, helps separate particles based on their size, density, or shape
MOI - Multiplicity of Infection this is the ratio of phages to bacteria An MOI of 01 means fewer phages compared to bacteria
TSP - (tryptic soy broth, nutrient-rich medium)
Lysed - the breakdown of a cell caused by damage to its plasma (outer) membrane
Supernatant - The soluble liquid reaction of a sample after Centrifugation or precipitation of insoluble solids
Smear Water - Water containing residue from food production, like in a dairy plant
PCR - Polymerase chain reaction, technique for rapidly producing (amplifying) millions to billions of copies of a specific segment of DNA, which can then be studied in greater detail

Objective
how environmental factors (chemical and physical) affect phage P100's ability to bind, replicate, and reduce L monocytogenes
Methods and Experiments

Adsorption Tests
Purpose - how well the phage (P100) binds to and infects bacteria under different conditions
Variables- Salinity pH (pH 2 and 12 excluded due to >7 log10 infectivity reduction within 1 hour)
Detergents (SDS, Lutensol AO 7), and Temperature
Steps

1. Adjust bacterial cultures to OD600 = 06 (optical density at 600 nm, a measure of how turbid (cloudy) the bacterial culture is, indicating the concentration of bacteria OD600 of 06 typically mean bacteria in the mid-log phase of growth are actively dividing)
2. Centrifuge and resuspended in TSB with test conditions phage added at MOI 01 the process of phage binding to bacteria is monitored by stopping the infection at specific

time points (eg, 0, 15, 30, 60 minutes) Ice-cold TSB halts phage activity and bacterial metabolism, preserving the state of the sample for further analysis

3. After separating the bacteria from the liquid (supernatant), the remaining free phages are counted using a double agar overlay plaque assay, a method where phages create clear zones (plaques) on a bacterial lawn, showing areas where bacteria have been lysed Replication measured at 0, 1, 3, 6, 12, and 24 hours (for pH 4)

Short-Term Chemical Influence -

- see how chemicals (like salt, detergents, and smear water) affect phage performance

Results - Some chemicals, like detergents, reduced the phage's ability to kill bacteria Smear water also made the phages less effective

Long-Term Temperature Influence - study how temperature affects phages over weeks Tested Temperatures - 4°C (fridge), 10°C (cool), 20°C (room temperature)

Results

1. At 4°C, the phages killed a lot of bacteria (7 log10 reduction, meaning they killed almost all bacteria)
2. After 2 weeks, the bacteria started growing back and became resistant

Resistance Screening - To check if bacteria developed resistance to the phage after long-term exposure

Results - Some bacteria became resistant to phage P100 resistance was confirmed using methods like

Phages Optimal Conditions

- Moderate pH, low salt, and clean environments help phages bind and kill bacteria effectively

Environmental Factors Can Reduce Phage Effectiveness

- Harsh conditions like extreme pH, detergents, or long-term storage make phages less effective
- Resistance Can Develop If bacteria are exposed to phages for too long without being completely killed, they can become resistant

Why Is This Important?

- helps scientists use phages more effectively in food production
- avoid conditions that make phages weaker or lead to resistant bacteria
- Phages could replace chemical preservatives and antibiotics in food safety
- Enhance food safety by controlling pathogens with phages
- Minimize chemical preservatives in food production
- Eco-friendly alternative to antibiotics

Future Research Directions

- Investigate synergistic effects of multiple phages
- Develop formulations to stabilize phages in adverse environments
- Test phages in real-world food production settings

Jan 11 2025

Notes I took from a previous paper I read (I took the notes myself previously but then had ChatGPT organize them better)
The notes are similar to our experiment, only also comparing adaptive evolution phages to wild-type phages and their performance in the different environments

Application of Adaptive Evolution to Improve the Stability of Bacteriophages during Storage

Read 1

Abstract

- A study compared the resistance of adapted phages (phages evolved to withstand stress) to ancestral wild phages under high-temperature conditions.
- Adapted phages demonstrated improved stability with minimal genomic changes.
- Adaptive evolution was utilized to improve resistance while maintaining key functions, such as lytic activity (the ability to infect and destroy bacterial cells).
- The process involved exposing phages to high-temperature cycles, allowing them to adapt and evolve, versus exposing wild-type phages directly to heat without adaptation.
- Experiments were conducted at 60°C (140°F).

Phages Used in the Experiment

- Pcc KPMI7
- Phage Wc4 (isolated from soil, China).
- Pba WHG10001
- Phage CX5 (isolated from freshwater, China).
- R. solanaceraum
- Phage P-PSG-11 (isolated from freshwater, Kenya).

Results

- Adapted phages survived better and had higher populations at the end of the experiment.

- Mutations occurred in:
- Tail tubular protein (proteins involved in phage structure and infection).
- Putative protein (a protein with an unknown function).
- Cytosine nucleotide (C in DNA: one of four building blocks of DNA) showed the most mappable mutations.

Read 2

Overview

- Focused on allowing phages to adapt to heat stress instead of transforming them through external modifications.
- Genomic changes in adapted phages were analyzed after adaptation cycles.

Steps to Create Adapted Phages

1. Heat treatment in a water bath at 60°C for 1 hour.
2. Cooling at 28°C (82.4°F) for 15 minutes.
3. Overnight incubation on agar plates (a gel medium for growing bacteria).
4. Centrifugation at 4°C (39.2°F) for 15 minutes to separate phages.
5. Repeated the water bath exposure (60°C for 1 hour).
6. This cycle was performed five times to induce adaptation.

Additional Experiments

- After adaptation, both ancestral and adapted phages were exposed to high temperatures (37°C/98.6°F) for 14, 30, and 60 days.
- Phage stability was measured using phage titers (the concentration of viable phages).
- Lytic activity was tested by monitoring bacterial growth over 12 hours.
- Experiments were repeated three times for consistency.

Temperature Selection

- 50°C: Minor titer decline (<1 log unit).
- 70°C: Complete titer loss.
- 60°C: Significant but survivable titer decline, making it the optimal stress level for adaptation.

Key Results

- Adapted phages cleared bacterial lawns (layers of bacteria on agar plates), indicating no significant loss of lytic activity.
- After five heat cycles:
- Adapted phages showed significantly less titer decline than ancestral phages.
- Examples:
- Pcc KPMI7 (Wc4 vs. Wc4-1): Titer loss reduced from 5.47 ± 0.25 log to 0.34 ± 0.13 log.
- Pba WHG10001 (CX5 vs. CX5-1): Titer loss reduced from 3.30 ± 0.36 log to 1.90 ± 0.20 log.
- R. solanaceraum (P-PSG-11 vs. P-PSG-11-1): Titer loss reduced from 3.60 ± 0.53 log to 1.29 ± 0.26 log.
- Long-term storage at 37°C for 60 days also showed lower titer reductions in adapted phages.

Phage Genomes

- Genome lengths remained consistent:
- Wc4/Wc4-1: 92,039 base pairs (bp) with 144 coding sequences (CDS) (regions that encode proteins).
- CX5/CX5-1: 43,885 bp with 55 CDS.
- P-PSG-11/P-PSG-11-1: 40,313 bp with 48 CDS.
- Genomic similarities:
- Wc4/Wc4-1: No significant similarity with other phages.
- CX5/CX5-1: 93.55% identity with Pectobacterium phage PP16.
- P-PSG-11/P-PSG-11-1: 94.86% identity with Ralstonia phage RsoP1EGY.

Mutations

- Mutations occurred only in coding regions.
- No insertions or deletions were observed.
- Examples:
- Wc4-1: Mutation in a putative protein (function unknown).

- CX5-1: Mutation in tail tubular protein gp12.
- P-PSG-11-1: Mutation in tail tubular protein gp11.

Conclusions

- Adaptive evolution improved phage stability under heat stress, but titer loss still occurred.
- This method needs refinement for greater resistance to fluctuating temperatures.
- Mutations were observed even in the ancestral phages, likely due to natural selection pressures.

References

Kering, K. K. (2020, April 9). Application of Adaptive Evolution to Improve the Stability of Bacteriophages during Storage. *Viruses*, *12*(4). https://doi.org/10.3390/v12040423

Jan 12 2025

- Started formulating hypothesis
- Wanting to use possibly these simulations ran with T4 phages as a basis
- https://medium.com/@IsavellaT/how-does-phage-therapy-actually-work-ba7ca8381e49
    - While not explicitly mentioned in the article because it wasn't relevant, the temperature and pH of the environment the simulations took place were
        - Temperature: 37C
        - pH: ~7.4
    - The temperature and pH of the environment are ideal for phages, especially the T4 phages we are working with, so it can provide a good baseline

Jan 15 2025

- Test temperatures
    - -10
    - 0
    - 10
    - **20**
    - **30**
    - **37**
    - **50**
    - 60
    - 70

- pH
    - 5
    - 6
    - 7
    - 8
    - 9

ROUGH PROCEDURE

1. Prepare the bacteria culture by implanting a colony of E.coli in Xml of LB broth
2. Incubate the bacteria overnight in incubator at 37°C
3. Get phage stock by spreading T4 phages to infect the E coli, centrifuge the culture and filter the supernatant through a 0.22 µm filter to obtain a phage stock, then store on ice
4. Prepare Ph buffer solution using phosphate buffered saline (PBS) or another buffer, use HCl or NaOH to adjust the pH of your solutions
5. From the previously prepared culture on day 1, dilute the E. coli culture to an OD600 of 0.1 using fresh LB broth
6. Dilute the T4 phage stock to the desired concentrations for infection(this is what i got fo dilution but idk 10^6, 10^7, and 10^8 pfu/mL)
7. Set up incubator
8. mix X mL of E. coli culture (OD600 = 0.1) with 1 mL of phage suspension
9. Get separate tubes for temperature and PH and make sure mixture is well mixed but don't shake too much
10. Incubate the phage-bacteria mixture for X amount of minutes at the designated temperature
11. While the infection is occurring prepare soft agar overlay
12. After the incubation period, add X mL of soft agar to the infected phage-bacteria mixture.
13. Pour this overlay mixture onto LB agar plates to form a solid overlay.
14. spread the mixture evenly and let it solidify at room temperature for X minutes.
15. Incubate the plates overnight for plaque formation
16. Count the number of plaques formed on each plate(You can calculate the phage titer (in pfu/mL) by multiplying the number of plaques by the dilution factor used for the phage stock, Repeat the procedure in triplicate for each temperature and pH condition to ensure reliable data.)

DRAFT 2 PROCEDURE

Here's your procedure, broken into clear sections with **materials listed in each step**. Any items you haven't specifically indicated you already have will be marked in **bold**. I've also included how to measure each quantity for clarity.

Day 1: Preparation

1. Prepare E. coli Culture

Materials:

- LB broth.
- *E. coli* bacterial culture.
- Test tubes or small flasks.
- Inoculation loop.
- Incubator set to 37°C.

Steps:

1. Use a sterile pipette to measure **5 mL of LB broth** and transfer it into a sterile test tube or flask.
    - **Measurement Tip:** Use a graduated pipette or micropipette for accurate measurements.
2. Sterilize an inoculation loop by flaming it over a burner, then allow it to cool.
3. Use the loop to transfer a single colony of *E. coli* into the LB broth.
4. Place the test tube or flask in the incubator at **37°C** and incubate overnight (12–16 hours).

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2. Prepare Phage Stock

Materials:

- LB broth.
- *E. coli* bacterial culture (from Step 1).
- T4 phages.
- **Centrifuge.**
- **0.22 µm filter.**
- **Sterile microcentrifuge tubes.**
- Ice.

Steps:

1. Measure **10 mL of LB broth** using a pipette and pour it into a sterile flask.
2. Add **1 mL of the overnight *E. coli* culture** to the LB broth.
3. Inoculate the mixture with **100 µL of T4 phages** using a micropipette.
4. Incubate the flask at **37°C** for 4–6 hours until the culture turns cloudy.
5. Centrifuge the culture at **5,000 × g for 10 minutes** to pellet bacterial cells.
    - **Note:** Ask your lab instructor to guide you in using the centrifuge.
6. Transfer the supernatant to a new sterile tube and filter it through a **0.22 µm filter** to remove bacterial debris.
7. Store the filtered phage stock on ice.

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3. Prepare Buffer Solutions for pH Testing

Materials:

- **Phosphate-buffered saline (PBS)** or sterile water.
- **HCl** (hydrochloric acid).
- **NaOH** (sodium hydroxide).
- Test tubes or flasks.
- pH meter or **pH strips**.

Steps:

1. Measure **10 mL of PBS** or sterile water using a graduated cylinder.
2. Add drops of **HCl** or **NaOH** to adjust the pH to desired levels (e.g., pH 5, 7, and 9).
    - **Measurement Tip:** Use a micropipette to add small, controlled amounts (1–10 µL) of acid or base.
3. Stir the solution thoroughly and check the pH with a **pH meter** or **pH strips**.
4. Prepare separate tubes for each pH level and label them.

Day 2: Testing

1. Dilute E. coli Culture

Materials:

- Overnight *E. coli* culture.
- LB broth.
- **Spectrophotometer.**
- Test tubes.

Steps:

1. Use a pipette to measure **1 mL of the overnight *E. coli* culture** and transfer it to a sterile test tube.
2. Measure the OD600 of the culture using a **spectrophotometer**.
3. Dilute the culture with fresh LB broth until the OD600 is **0.1**.
    - **Example**: Add 1 mL of culture to 9 mL of LB broth and mix.

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2. Dilute the Phage Stock

Materials:

- Phage stock (prepared on Day 1).
- Sterile water.
- Sterile test tubes.
- Micropipette.

Steps:

1. Label sterile test tubes for dilutions (e.g., 10^6, 10^7, 10^8 PFU/mL).
2. Add **900 µL of sterile water** to each tube using a micropipette.
3. Add **100 µL of phage stock** to the first tube (10^6) and mix by gently pipetting up and down.
4. Take **100 µL from the first tube** and transfer it to the second tube (10^7).
5. Repeat for subsequent dilutions.

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3. Set Up pH Testing

Materials:

- Diluted *E. coli* culture (OD600 = 0.1).
- Diluted phage stock.
- Buffer solutions (prepared on Day 1).
- Test tubes.

Steps:

1. Add **1 mL of *E. coli* culture** to each tube containing a pH buffer solution.
2. Add **1 mL of phage stock** to each tube.
3. Mix gently to combine, avoiding bubbles.

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4. Set Up Temperature Testing

Materials:

- Diluted *E. coli* culture (OD600 = 0.1).
- Diluted phage stock.
- Test tubes.
- Incubator.

Steps:

1. Add **1 mL of *E. coli* culture** to each test tube labeled with the desired temperature (e.g., 25°C, 37°C, 45°C).
2. Add **1 mL of phage stock** to each tube.
3. Mix gently and place the tubes in the appropriate incubator or temperature-controlled environment.

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5. Prepare Soft Agar Overlay

Materials:

- Agar powder.
- LB broth.
- Water bath or **microwave**.

Steps:

1. Heat the agar mixture (0.7% agar in LB broth) in a water bath or **microwave** until fully dissolved.
2. Keep the soft agar at **~45°C** to prevent it from solidifying.

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6. Plate the Samples

Materials:

- Pre-poured nutrient agar plates.
- Soft agar.
- Phage-bacteria mixtures.

Steps:

1. Add **3 mL of soft agar** to each tube of phage-bacteria mixture.
2. Mix gently and pour onto labeled nutrient agar plates.
3. Spread the mixture evenly by tilting the plates and let them solidify at room temperature for ~10 minutes.

Day 3: Data Collection

Count Plaques

Materials:

- Incubated plates.
- Marker.
- Calculator (optional).

Steps:

1. Examine the plates for plaques (clear spots).
2. Count the plaques on plates with 30–300 plaques.
3. Use the formula: Phage Titer (PFU/mL) = (Number of Plaques × Dilution Factor)/[Volume Plated (mL)]

Jan 16 2025

- Emailed MRU for access to a lab

Jan 17 2025

- Emailed 2 more people
- Sample email:

Hello [name],
I'm Isavella and I'm also emailing on behalf of my partner, Serena, for the Calgary Youth Science Fair. For our science fair project, we were looking into conducting a variety of experiments to test the infectivity/effectivity of T4 bacteriophages in different environments varying in temperature and pH. In order to perform this experiment, there are a few resources that we do not have access to, as well as some that we can't obtain on our own. If we were to try to get these materials on our own, it would either be inaccessible to us or it would become too much of a cost. Therefore, it would be greatly appreciated if we could have access to one of your labs to perform our experiments. Some materials we need include:
Access to an incubator (so that we can perform the temperature tests accurately, as well as preserve our phages/bacteria at proper temperatures)
HCl, NaOH, Phosphate-Buffered Saline (PBS), pH strips/meter (to change the pH of the environment the phages will be infecting the bacteria in)
Standard lab equipment (petri dishes, nutrient agar/soft agar, sterile water, inoculation loops, pipettes, pH strips/meter, spectrophotometer, 0.22 µm Sterile Filters)
Again, it would be greatly appreciated if we could have access to your lab and this equipment. Being able to do something like this, while incorporating interests we are so passionate about, would be both a unique and educational experience for us.
It would be best for us to have access to your lab end of January/beginning of February.
Please let us know what we can do.
Looking forward to hearing from you,
Isavella and Serena

Jan 20 2025

- Notes taken for two more papers that are testing for the environmental stability of phages
- Heard back from MRU and said they would look into our request

Isolation, Characterization, and Genome Engineering of a Lytic Pseudomonas aeruginosa Phage

Study: Isolation, Characterization, and Genome Engineering of a Lytic Pseudomonas aeruginosa Phage

1. Background

- Problem: Surge of multidrug-resistant Pseudomonas aeruginosa strains.
- Phage Identified: PpY1
- Phage Description:
- Short tail.
- Genome length: 43,787 base pairs (bp).
- Objective: Reduce the phage genome by eliminating non-essential genes.
- Phage Behavior: Forms biofilms (clusters of bacteria surrounded by a sticky protective layer).

2. Phage Isolation and Culturing

- Phage Isolation:
- Found in feces from a pig farm.
- Incubation: Incubated at 37°C overnight.
- Culture Method: Phage was cultured using the double agar method (a technique for growing bacteria and observing phages).
- Centrifugation: Phage particles were separated using centrifugation (spinning at high speeds) and filtered.

3. Stability Testing

- Temperature Endurance: Phage was tested for stability at different temperatures for 1 hour each:
- 20°C, 30°C, 40°C, 50°C, 60°C, 70°C.
- pH Endurance: Phage tested for stability in different pH (acidic or basic environments) for 1 hour each:
- pH 3, 5, 7, 9, 11, 13 (using NaOH or HCl to adjust pH).
- Results:
- pH:
- Active between pH 5 and 7.
- No activity at pH 3.
- Titer (quantity) decreased at pH 13.

- Temperature:
- Stable between 20°C-50°C.
- Optimal at 40°C.
- No activity at 70°C.

4. Lytic Activity Test

- Lytic Activity: Phage tested for lytic activity (ability to destroy bacteria) against different P. aeruginosa strains.
- Spread onto bacterial plates and incubated at 37°C overnight.
- Growth Curve: The phage's growth was tracked over time:
- Latent phase: Approximately 15 minutes (the period after infection but before the phage starts multiplying).
- Burst phase: 45 minutes (phages are rapidly produced and released from infected cells).
- Plateau: Phage titer (concentration) reached 10^{13} plaque-forming units (PFU) per milliliter after 60 minutes.

5. Genome Sequencing and Features

- Genome Sequencing:
- Sequenced using Illumina NovaSeq and Oxford Nanopore (ONT).
- Genome Features:
- Length: 43,787 bp.
- GC Content: 62.2% (percentage of the genome composed of guanine and cytosine nucleotides).
- Two direct terminal repeats (DTRs) of 440 bp at both ends.
- T7-like tail components identified: proteins such as head-to-tail connector (Gp8), tail tubular protein (Gp11), tail fiber protein (Gp17), suggesting that the phage has similar characteristics to the T7 phage family.
- Lytic Nature: No lysogeny modules (genes responsible for integrating the phage's DNA into the host's genome), confirming that PpY1 is purely lytic (kills the bacteria it infects).
- 58 potential genes identified, 27 had known functions, and 30 were hypothetical proteins (no known function).

6. Genome Engineering and Gene Deletion

- Goal: Reduce genome size by eliminating non-essential genes.
- 21 non-essential genes were successfully deleted.
- Gene Deletion Effects:
- No impact on the phage's assembly.
- Some deletions affected the size of plaques (visible spots where phages destroyed bacterial cells), the timing of lysis (when bacteria are killed by the phages), and phage titer (concentration).
- Plasmid Assembly:
- Phage genome assembly attempted in E. coli (bacterial species), but failed.
- Successful Assembly: The phage genome was successfully assembled in Saccharomyces cerevisiae (a type of yeast), resulting in 29 distinct genomes.
- Plasmids (DNA molecules) with the phage genomes were extracted from yeast and introduced into P. aeruginosa (bacterial species), but RM (restriction-modification) systems in the bacteria interfered with DNA delivery.
- The problem was solved by using a strain of P. aeruginosa (PAO1-KORM) that lacked the RM system, allowing the genome delivery to work.

7. Genetic Stability

- Stability Test: The modified phages were passed through 10 generations to test their genetic stability.
- Lytic Activity:
- The wild-type phage (original version) had lytic activity around 130 minutes after infection.
- Modified phages had varying lysis times from 130 to 160 minutes.
- Gene Deletion Effects:
- The deletions affected the timing of lysis and plaque size but didn't affect the phage's ability to infect all 26 tested strains of P. aeruginosa.

8. Gene Editing Method

- Targeted Cloning:
- TAR cloning (Targeted Recombination-Associated Cloning) was used instead of large-scale CRISPR-Cas9 genome editing.

- Gene deletions did not significantly impact plaque formation, but some affected the size of plaques, phage titer (concentration), or lysis timing.

Key Terms Defined:

- Biofilms: Clusters of bacteria protected by a sticky substance, which makes them harder to kill.
- Centrifugation: A process that spins liquid samples at high speeds to separate components based on density.
- Phage Titer: A measure of the concentration of phages in a solution, typically represented as the number of plaque-forming units per milliliter (PFU/mL).
- Plaque-Forming Units (PFU): The number of distinct areas (plaques) formed when phages infect and destroy bacterial cells on an agar plate.
- GC Content: The percentage of the DNA made up of guanine (G) and cytosine (C) bases, important for understanding the stability and structure of the genome.
- Lysis: The breaking open of a bacterial cell, often caused by the action of phages that infect and multiply inside the cell.
- Plasmids: Small DNA molecules found in bacteria, separate from their chromosomal DNA, which can carry genetic material.
- Restriction-Modification (RM) System: A bacterial defense system that recognizes and cuts foreign DNA (such as phage genomes).

- Pseudomonas aeruginosa phage has shown a surge of multidrug-resistant strains
- The phage
  - PpY1
  - Short tail
  - 43787 bp genome length
- Trying to reduce phage genomes
- Eliminated 21 non-essential genes from the genome, caused more of those phages to have reduced genomes
- The PpY1 phage creates biofilms
- The problems of not knowing about what the uses of phage genes are, restricted genome space and alterations for more applications and capabilities
- Sequenced the genome and identified useless genes
- The phage strain was found at a pig farm from feces
- Incubated at 37C overnight
- Centrifuged and filtered, then cultured using the double agar method
- They tested temperature and pH endurance
  - 1 hr in each of the temperatures
    - 20C
    - 30C

        - 40C
        - 50C
        - 60C
        - 70C
    - 1 hr in each pH environment used NaOH or HCl
        - 3
        - 5
        - 7
        - 9
        - 11
        - 13
- They tested the lytic activity of the PpY1 phage against different P. aeruginosa strains by being spread on the cultured plates of bacteria and incubated at 37C overnight
- To extract the phage genomes
    - Were injected with growing cells for said strain, along with DNasel and RNaseA, then incubated at 30C for 30 mins
    - Solid NaCl was added and then stirred till dissolved
    - Then was placed in an ice bath for 1 hr
    - Cell debris was removed by centrifugation for 10 mins at 4C
    - The mixture was inverted gently
    - Then incubated for 1 hr at 50C
    - The DNA extraction solution was added then the mixture was fully emulsified
    - The emulsion was centrifuged for 30 mins
    - Created DNA percipiates
    - Centrifuged for 3 mins
    - Pellets were air-dried
    - Finally dissolved in ddH2O
- Genome sequenced with Illumina NovaSeq and Oxford Nanopore ONT techniques
- Genes were annotated with RAST server
- The bacteria were infected with the genome-edited phages and monitored the populations and infection rates
- The one-step growth curve analysis indicated that PpY1 has a latent phase of approximately 15 min, followed by a burst phase lasting 45 min. The phage titer reached a plateau at around 1013 plaque-forming units (PFU) per milliliter after 60 min
- Graph features one-step growth curve of phage PpY1, showing average phage titers from three independent cultures.
- Results from temperature and pH experiments
    - Results for pH
        - Significant biological activity at pH 5-7
        - decline of phage titer at exposure to pH 13
        - and no plaques were detected at pH 3
    - Results for temperature
        - Relatively stable phage titers at temperatures 20C-50C
        - optimal at 40C
        - Significant titer reduction at 60C
        - No plaques were observed at 70C

- PpY1, which showed potent lytic activity against some of the 26 strains of P. aeruginosa, including those resistant to traditional antibiotic treatments
- Sequenced PpY1 genome
    - Length of 43787bp
    - Average C+G of 62.2%
    - Two direct terminal repeats (DTRs) of 440bp at both ends
    - We also identified several T7-like tail components within the PpY1 genome, including T7-like proteins like head-to-tail connector (Gp8), the tail tubular protein (Gp11), and the tail fiber protein (Gp17). These suggest that PpY1 may share similar functional characteristics with the T7-like phages
    - did not reveal any lysogeny modules or lysogens (**Table 2**), confirming that phage PpY1 is indeed lytic in nature
    - we predicted 58 ORFs encoding proteins with at least 30 amino acids as potential genes (*gp01-gp58*, **Figure 3**). Among these, 27 gene products showed a significant homology to proteins with known functions. The remaining 30 genes, which did not show a significant homology to any known proteins, are hypothesized to encode hypothetical proteins
- Attempted to assemble PpY1 phage genome in E. Coli, but failed
- S. cerevisiae was the host for phage genome assembly
- 29 distinct genomes were successfully assembled
- Plasmids with varied PpY1 genomes were extracted from yeast and introduced into the P. aeruginosa strain (PAO1)
    - No plaques formed due to the potential interference of restriction-modification (RM) systems in DNA delivery
    - Looked through the PAO1 genome for the gene
    - Found an RM system-deficient strain, PAO1-KORM, and used this instead which worked
- The gene alterations performed on the wild phages, and 21 gene modifications were successful and had no impact on the phage assembly process or stability
- Reviewed genetic stability by passing it through 10 generations
- PpY1 had lytic activity at around 130 mins, while variations had faster ones to longer ones of up to 160mins
- Some of the genes deleted may impact the timing of lysis activity and plaque formation size
- The mutated phages had varying results in being able to infect all 26 P. aeruginosa strains
- Instead of using large-scale CRISPR-Cas editing, they used a targeted TAR cloning approach
    - While the individual deletion of these nonessential genes did not impact plaque formation, some affected plaque size, phage titer, or the timing of cell lysis

Genetically manipulated phages with improved pH resistance for oral administration in veterinary medicine | Scientific Reports

- Phages that are orally administered in veterinary cases face several challenges such as
    - Temperature
    - Salinity
    - *Primarily* pH
- Encapsulation had some success, but is too complex
- Improved stability of the mutant phages had survival rates of 10^2-10^7 pfu.ml^-1, higher than wild type phages
- Low pH values significantly reduce phage titer
- Temperatures of animals in their body can also affect treatment efficacy
    - Temperatures above optimum prolong phage's latent period
    - Below optimum reduce phage penetration and consequent proliferation
- Different enzymes like pepsin, amylase, lipase, and protease may also cause degradation of phages
- Developing gene editing technique for phages like BRED makes it cheaper and more appealing to conduct gene editing on phages to combat this issue rather than the past strategy of encapsulation of phages
- E. Coli T7 phage was model, modified the genome with E. coli outer membrane phosphoporin protein E (PhoE) signal peptide
    - Thought to be directly involved in the binding of proteins to the membrane by electrostatic interactions between the n-terminus and anionic membrane phospholipids
- Lipid coating of phages through genetic engineering all in all maybe be a promising alternative to combat different environments
- Infused E. coli PhoE signal peptide to major capsid protein (gp10A) using E. coli phage T7 as a model phage
- BRED was used to insert PhoE signal peptide into T7 genome, fused to major capside protein, obtaining mutant phage T7::PhoE
- Mutation remained stable for at least 10 generations
- Infectivity of mutant phages was compared to wild type phages
    - Assessed through 1 step growth curve
    - Latent period established at approx. 15 mins
    - Eclipse time of 10 mins
    - Burst size of 253±30 for T7 and 243±92 for T7::PhoE
    - Identical with no effect of the mutation on phage replication
- Attempt to visualize presence of coating surface around mutated phages
- No clear differences could be seen
- Other methods were used such as staining, but none indicated presence of lipid coating on mutant phages
- Analyzed on HPTLC plates, and separated lipids were visualized and shows an increased lipid content of the mutant phages
- Charge of phage particles were significantly different (P=0.0154)

    - Average values of -5.49mV and -8.69mV for T7 and T7::PhoE
- Signal peptide had predicted positive net charge of +2mV
- Phages used in in vitro survival assays simulating different conditions encountered along the GIT of animals (the focus environment for this study)
- Temperatures ranged from 38C to 42C to represent typical body temperatures of different animals
- pH levels ranged from 2.5 to 5.7 to simulate acidic environments found in compartments of the GIT
- Time ranged from 15 to 1440 mins (24h) (residence times of compartments in GIT)
- Results
    - Most severe condition for pH (2.5)
        - Wild type phage titer lost completely in just 15 mins
        - Mutant phage survives up to 30 mins with 10^6-fold decrease in titer
    - pH values 3.5, 4.5, and 5.7
        - Mutant phage titer generally decreased by factor of 10^1 or 10^2
        - Wild type phage titer generally decreased by factor 10^2 and 10^4
        - 100 fold different can have significant impact on the course of veterinary oral phage therapy
- For oral application success of phages, doses need to be as high as 10^11-10^13 pfus.ml^-1 to ensure significant number of phages (at least 10^6) reach the intestines (GIT area around)
- Strong differences in mutant vs wild type phages were observed in different temperatures
    - Particularly for pH 3.5 at temperature 42C where 10^4-10^7 difference could be observed after 180 mins and 1440 mins of incubation
- Only the capsid is modified by the mutation, not the tail or fibres, which suggests that the pH and varying temperatures mainly effects the genetic material of the phage in the capsule, because they are still infecting in the varying environments even better than before
- It was tested to ensure they had the capacity to multiply after being subjected to the acidic environment after the mutation of only the capsule
    - After 3h or incubation with bacterial host, phage titer increased 100-fold
    - Indicates phages are still able to infect and multiply
- Tested the survival of the mutated phages by using enzymes able to specifically degrade phospholipids (like the PhoE signals added to the capsid for protection), phospholipases
    - Both phage types were subjected to phospholipase A2, which recognizes and hydrolyses the sn02 acyl bond of phospholipids, forming a fatty acid and a lysophospholipid
    - Mutant phage represents log survival similar to wild type phage and lower to it as well
    - Further confirms presence of phospholipids on the capsid of the mutant, while indicating involvement on pH tolerance (low pH mainly looked at)
    - The phospholipase A2, for example, is located in pancreatic fluids in the gut, an could revert the effect of the phospholipids on phage capsids for the mutant phages

    - But it is known that once in the gut, the phages will not be exposed to the low pH values, so they do not require that additional protection of the phospholipids
    - The treatment therefore will not be compromised
- The phages will also need resistance to different hydrolytic enzymes such as pepsin, lipase, amylase, and protease
    - The stability of the 2 phages types were tested under the simulated conditions
    - The pancreatic enzymes (such as protease) within range concentration found in duodenal juice (around 1.4 ± o.7 mg/ml) had no major effect on viability of wild type and mutant phages
    - although significant changes (P<0.05) were found after 120 mins of incubation
    - In general pancreatic enzymes did not appear to be major concerns for the oral delivery of phage T7, with less than 55 reduction in phage titer
    - Gastric enzymes led to substantial reduction in number of wild type t7 at 90 mins and 120 mins
    - Mutant T7::PhoE remained mostly unaffected (P<o.o5)
    - The presence of lipids/phospholipids may restrict the access of those enzymes to the phage and prevent degradation
- The protection of the lipid coating seems to be similar or even superior to that of the encapsulation approaches
- Bile acid (secreted into the GIT where it works as an anionic surfactant) has an affect on phages, so it was tested the stability between wild type T7 phages and mutant T7::PhoE phages
    - Wild type phages were affected significantly more (P<o.o5) than the mutant phages after 1 hr incubation
    - They have similar affects after 3 hrs with viability reduction of about 16% 9corresponding to 1long)
    - Bile concentration was of 2%, which exceeds to that found in bile produced in the organism (around 0.7%)
    - Affects are expected to be lower than observed here
- Insertion of PhoE capsid signal of phage T7 allows future exploitation of the frameshifting on gene 10
    - Characterised by the expression of 2 products, major 10A protein, minor 10a protein, resulting in different phage phenotypes
    - Replication of mutant T7::PhoE in other hosts (different E. coli strains) could result in changing the copy number of 10A, resulting in different phenotypes of the same mutant
- The mucus rich environments in the GIT has been proven to be the principal location of most translocation events of bacterial pathogens
    - Future in vivo studies can test ability of the mutant phage to survive longer periods in the gut by fast absorption to and diffusion into the mucosa, increasing encounters with pathogenic host and maybe improving efficacy of phage therapy
- Buffers and media

    - Saline-Magnesium (SM) buffer, containing 100 mM NaCl, 8 mM $MgSO_4.7H_2O$ and 50 mM Tris (pH 7.5) was used for phage dilutions
    - Lysogeny broth (LB) medium[54] was used for bacterial growth
    - Super Optimal broth with Catabolite repression (SOC) was used to recover cells after recombineering
    - Agar was used at a concentration of 1.2% in plates and 0.7% in soft agar
    - Ampicillin (Amp) was used at a final concentration of 100 $\mu g.mL^{-1}$
    - L-arabinose at 10% (w/v in water) was used to induce recombineering functions
- Phages, bacteria, and plasmids
    - Phage host was E. coli BL21
    - Plasmid pKD46, ampicillin resistant and temperature sensitive plasmid, encodes lambda red genes, was used to prepare recombineering competent cells
- Preparation of recombineering competent E. coli BL21 cells
    - cells were grown in LB medium to mid-log phase and harvested by centrifugation (3000 × *g*, 4 °C, 15 min)
    - The culture was concentrated 500-fold by washing three times with ice-cold sterile 10% (v/v) glycerol, and suspended in a final volume of 80–300 µL
    - Then, 100–500 ng of pKD46 were electroporated into aliquots of 20 µL electrocompetent cells at 1.8 kV and 25 pF using a Gene Pulser XCell Microbial System (BioRad)
    - Cells were recovered with SOC and incubated for 1–2 h at 37 °C, 200 rpm
    - Cells were spread in LB plates containing ampicillin (LB-Amp) and incubated overnight at 30 °C
    - Transformed *E. coli* BL21::pKD46 colonies were picked and grown in fresh LB-Amp medium for a few hours, followed by plasmid extraction and digestion with the restriction enzyme BamHI-HF to confirm the presence of pKD46
- Construction of recombineering substrates
    - the primers were used at a concentration of 25 µM for Knight - annealing and primer extension with Taq polymerase
    - The forward and reverse primers of the PhoE signal peptide were mixed with water, 10x buffer Taq A, dNTPs and Kapa Taq

DNA polymerase, and annealed and extended using the following program:
        - 95 °C for 5 min; 5 cycles of 95 °C for 30 s, 50 °C for 30 s, and 72 °C for 30 s; and 72 °C for 5 min
    - The resultant constructs were purified using the GRS PCR & Gel Band Purification Kit (Grisp) and sizes confirmed on a 1% SGTB agarose (Grisp) gel using the Low Molecular Weight DNA ladder
- Phage recombineering of electroporated DNA
    - Briefly, *E. coli* BL21::pKD46 was grown to early-log phase at 30 °C in 100 mL LB-Amp medium, and the expression of lambda Red by pKD46 was induced for 30 min with L-arabinose (0.1% final concentration)
    - Cells were then infected with T7 at a multiplicity of infection (MOI) of 1–3 for the eclipse time (10 min) (making them electrocompetent)
    - 100–500 ng of the substrate were electroporated into the electrocompetent cells at 1.8 kV and 25 pF, and cells were recovered with SOC medium for 1–2 h at 37 °C, 200 rpm
    - The suspension was then mixed with approximately 3 mL soft agar and 100 µL of an *E. coli* BL21 culture, poured onto LB plates, and incubated overnight at 37 °C
- Recovery and confirmation of mutant phages
    - Phage plaques obtained were assessed for presence of mutants
    - Several phage plaques were picked and replicated in E coli BL21 for 2-3 hrs
    - Phages were recovered by addition of chloroform, followed by centrifugation at 12000 x g for 15 mins, and collection of the supernatant
    - Extracted using Proteinase k at 37C for 40 mins, followed by inactivation at 100C for 15 mins
    - Confirmation primers were used in reaction volumes of 25 µL and temperature of 50C
- Phage purification by cesium chloride density gradient centrifugation
    - A step gradient of cesium chloride solutions (5.7 mL) was layered on Beckman centrifuge tubes, from the least to the most dense (ρ = 1.33, 1.45, 1.50 and 1.70 g.mL^{-1}), adding each new layer to the bottom of the tube
    - The phage solution (15.2 mL containing 0.5 g of cesium chloride per mL of solution to avoid osmotic shock) was carefully layered over the gradient

    - The tubes were centrifuged at 28,000 × *g* for 3 h at 4 °C (Beckman Optima Ultracentrifuge XL-80K) in a SW28 rotor
    - After centrifuging, the band formed by the purified phage was recovered using a syringe with a 20 gauge needle to perforate the side of the centrifuge tubes
    - Cesium chloride was removed by dialysis against SM buffer at 4 °C
- Can look at paper for rest of methods to answer any questions about in depth procedures

References

Cong, X. (2024, November 16). Isolation, Characterization, and Genome Engineering of a Lytic Pseudomonas aeruginosa Phage. *Microorganisms*, *12*(11). https://doi.org/10.3390/microorganisms12112346

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Jan 22 2025

Updated procedure:

Updated Procedure for Testing Phage Effectiveness Under Different pH and Temperature Conditions

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Day 1: Preparation

1. Prepare E. coli Culture from Agar Plate

Materials:

- *E. coli* agar culture (from phage set).
- LB broth (5 mL per replicate).
- Sterile inoculation loop.
- Sterile test tubes.

Steps:

1. Using a sterile inoculation loop, pick a single colony of *E. coli* from the agar plate.
2. Transfer the colony to a sterile test tube containing **5 mL of LB broth**.
3. Mix gently to suspend the bacteria.
4. This suspension is now ready for OD600 measurement and use in subsequent steps.

---

2. Dilute E. coli Culture to OD600 = 0.1

Materials:

- *E. coli* suspension prepared above.
- Spectrophotometer.
- Sterile LB broth.
- Sterile cuvettes.
- Micropipettes and sterile tips.

Steps:

1. Turn on the spectrophotometer and set it to **600 nm** wavelength.
2. Blank the spectrophotometer using a cuvette filled with sterile LB broth.
3. Measure the OD600 of the *E. coli* suspension.
4. If the OD600 is higher than 0.1, dilute the suspension:
    - Use the formula: $\mathbf{V_1 C_1 = V_2 C_2}$ - Example: To prepare 10 mL of culture at OD600 = 0.1 from OD600 = 1.0, mix 1 mL of the original culture with 9 mL of LB broth.
5. Verify the OD600 of the diluted culture.

---

3. Prepare Phage Dilutions

Materials:

- T4 phage stock (pre-prepared).
- Sterile water.
- Sterile test tubes.
- Micropipettes and sterile tips.

Steps:

1. Label sterile test tubes with dilution factors (e.g., 10^6, 10^7, 10^8).
2. Add **900 µL of sterile water** to each tube.
3. Add **100 µL of the phage stock** to the first tube (10^6) and mix gently.
4. Transfer **100 µL** from the first tube to the second tube (10^7) and repeat for the subsequent tubes.

---

4. Prepare Buffer Solutions for pH Testing

Materials:

- Phosphate-buffered saline (PBS) or sterile water.
- HCl (hydrochloric acid).
- NaOH (sodium hydroxide).
- pH strips or pH meter.
- Sterile test tubes.

Steps:

1. Prepare **PBS or sterile water** solutions for pH adjustment.
2. Adjust the pH to desired levels (e.g., pH 5, 7, 9) using HCl to lower the pH and NaOH to raise it.
3. Verify the pH using pH strips or a pH meter.
4. Label each buffer solution.

Day 2: Testing

1. pH Testing Setup

Materials:

- Diluted *E. coli* culture (OD600 = 0.1).
- Diluted phage stock.
- Prepared buffer solutions.
- Sterile test tubes.

Steps:

1. Label test tubes for each pH condition (e.g., pH 5, pH 7, pH 9).
2. Add **1 mL of diluted *E. coli* culture** to each tube.
3. Add **1 mL of phage stock (e.g., 10^7 PFU/mL)** to each tube.
4. Add **1 mL of the corresponding buffer solution** to each tube.
5. Mix gently by pipetting up and down.

---

2. Temperature Testing Setup

Materials:

- Diluted *E. coli* culture (OD600 = 0.1).
- Diluted phage stock.
- Sterile test tubes.
- Incubators or temperature-controlled setups (e.g., 25°C, 37°C, 45°C).

Steps:

1. Label test tubes for each temperature condition (e.g., 25°C, 37°C, 45°C).
2. Add **1 mL of diluted *E. coli* culture** to each tube.
3. Add **1 mL of phage stock (e.g., 10^7 PFU/mL)** to each tube.
4. Mix gently by pipetting up and down.
5. Place the tubes in the corresponding incubator or temperature setup for **30 minutes**.

---

3. Prepare Soft Agar Overlay

Materials:

- Agar powder (0.7% agar solution in LB broth).
- Water bath or microwave.

Steps:

1. Dissolve agar powder in LB broth to make a 0.7% solution.
2. Heat the solution in a microwave or water bath until fully dissolved.
3. Maintain the soft agar at **~45°C** to keep it molten.

---

4. Plate the Samples

Materials:

- Pre-poured nutrient agar plates.
- Phage-bacteria mixtures.
- Soft agar overlay.
- Sterile pipettes.

Steps:

1. Add **3 mL of soft agar overlay** to each tube containing the phage-bacteria mixture.
2. Mix gently and pour the mixture onto pre-labeled nutrient agar plates.
3. Tilt the plates to spread the overlay evenly.
4. Let the plates solidify at room temperature for **10 minutes**.

Day 3: Data Collection

1. Count Plaques

Materials:

- Incubated agar plates.
- Marker or magnifying glass.
- Calculator (optional).

Steps:

1. Examine the plates for plaques (clear zones). Each plaque represents an area where a phage has lysed bacteria.
2. Count plaques on plates with **30–300 plaques**, as this range provides accurate data.
3. Record the plaque counts for each condition.

---

2. Calculate Phage Titer

Formula:

Phage Titer (PFU/mL)=Number of Plaques×Dilution FactorVolume Plated (mL)\text{Phage Titer (PFU/mL)} = \frac{\text{Number of Plaques} \times \text{Dilution Factor}}{\text{Volume Plated (mL)}}

Example:

- If 50 plaques are observed on a plate from the 10^7 dilution, and 0.1 mL was plated:

Phage Titer=50×1070.1=5×109 PFU/mL.\text{Phage Titer} = \frac{50 \times 10^7}{0.1} = 5 \times 10^9 \text{ PFU/mL.}

---

3. Repeat for Triplicates

- Perform the experiment in triplicate for each pH and temperature condition to ensure reliable data.
- Average the results for final analysis.

Final Notes

- **Aseptic Techniques**: Maintain sterility at all times to avoid contamination.
- **Consistency**: Use the same amounts and conditions for all tests to ensure comparability.
- **Safety**: Handle all materials, including HCl and NaOH, with proper safety equipment (e.g., gloves, goggles).

Jan 28 2025

- Have an in-person date set: Feb 15th
- Going to work on research separately and then review it together
- Research questions
    - Evolution of phage therapy from advantages to obstacles?
    - The different applications and research areas in phage therapy?
    - The innovation in the field?
    - What is it and how does it work?

    - How does identifying the stability of phages in various environments help overcome the obstacles in implementing phage therapy-related techniques?
    - What are the current optimal environments for phages to thrive?

Feb 5 2025

https//wwwncbinlmnihgov/books/NBK493185/

Phages (basic info)

- Viruses that only infect a single bacterial species or even specific strains within a species
- Head with nucleic acid genome in a capsid protein shell; non-motile, depend on Brownian motion for movement
- Uses 2 types of replication strategies

Lytic

- Phage infects bacterium, uses host's ribosomes to manufacture its proteins to create new phages
- Host cell lyses, releasing new phages to infect others

Lysogenic

- introduces its genome into the host cell cytoplasm
- Phage genome integrates into host's chromosome or maintained as an episomal element as prophage
- Bacteria (lysogens) replicate phage genome without killing the cell
- Prophages can switch to lytic cycle under stress

Bacteriophages & Human Microbiome

- Cannot infect or replicate in human cells
- Important in human microbiome, mediates genetic exchange between pathogenic and nonpathogenic bacteria
- Transduction Transfer of genes between bacterial strains by phages

Types of Transduction

Generalized Transduction

- Random bacterial DNA packaged into phage capsids during lytic replication
- Phage injects bacterial DNA into new host, integrating into the host's genome
- genetic changes in the host and its offspring (eg, antibiotic resistance)

Specialized Transduction

- Lysogenic phages - Integrate into bacterial genome without killing host
- When switching to lytic cycle, phage excises bacterial DNA along with its genome
- Transduces specific bacterial genes to new hosts, often including virulence factors

Phages attack specific bacterial species/strains, altering microbial populations

Phage Therapy Using phages to treat bacterial infections in humans and animals

Challenges

Bacterial resistance Some bacteria resist phages, similar to antibiotic resistance (eg, CRISPR-Cas9 system)

Immunogenicity Phages are large and trigger immune response, cleared rapidly from the blood

Limited use Phages more suited for topical use, not systemic due to immune clearance

No proven efficacy No randomized controlled trials confirming phage therapy effectiveness in humans

Alternatives

Phage enzymes Enzymes from phages that break down bacterial cell walls may be simpler for bacterial infection treatment

https//journalsasmorg/doi/pdf/101128/jvi01821-23

Phage classification

Phage Classification Methods

- Infection Cycle – How phages replicate and interact with bacterial hosts
- Morphology – Structural traits like shape, size, and tail type
- Taxonomy – Genetic relationships and evolutionary lineage
- Supergroups – Broad genomic and functional classifications
- Phage Classification by Infection Cycle

Plaque Formation

Plaques (clear or turbid zones) help identify phages

Not all phages form plaques, but plaque assays widely used

Types of Infection Cycles

Lytic Cycle (Virulent Phages)

- Phage immediately takes over host, replicates, and lyses (destroys) it

Produces clear plaques (zones of bacterial cell death)

Examples T4, T7 phages

Lysogenic Cycle (Temperate Phages)

- Phage DNA integrates into the bacterial genome as a prophage

Host cell (lysogen) survives and divides with the prophage DNA

Can switch to the lytic cycle under stress

creates turbid plaques (unclear/cloudy zones)

Well-studied examples Lambda, Mu, P22, P1 phages

Chronic Infection Cycle (Filamentous Phages)

- Phages replicate continuously without lysing the host
- Virions are secreted from intact cells
- Difficult to distinguish from lysogenic cycles

Example - M13 phage

Phage Terminology

- Virulent Phages -> Only use the lytic cycle (eg, T4, T7)
- Temperate Phages -> Can switch between lysogenic and lytic cycles (eg, Lambda, Mu)
- Lytic Phage (often used for virulent phages) means the phage always lyses the host
- Lysogenic Phage can refer to either a temperate phage in the lysogenic cycle or a lysogen (host cell with a prophage)
- Chronic Infection differs from the lysogenic cycle as phage replication is continuous, whereas lysogenic phages remain mostly dormant

Research and Classification Tools

- Recent reviews provide deeper insights into infection cycle characteristics

- When only sequence data is available, phages can still be classified into infection cycles using computational methods

predict phage infection cycle type

- Horizontal gene transfer rates (HGT)
- Genome comparisons between phage and host
- Presence of hallmark proteins (key conserved proteins linked to infection cycles)

Hallmark Proteins in Phage Classification

- Indicator proteins related to infection cycle type and predict phage behavior

Databases of hallmark proteins help classify phages

- Temperate Phages → Contain
- (used for genome integration/excision)
- Virulent Phages → Lack these integration-related genes
- Filamentous Phages → Identified by specific hallmark protein patterns

Major Phage Morphotypes

Non-tailed Phages

- Pleomorphic – Shape varies, sometimes enveloped (e.g., phi6).
- Filamentous – Long, thread-like (e.g., M13).
- Polyhedral/Icosahedral – Geometric, often small (e.g., phiX174, QBeta).

Tailed Phages (Caudovirales Order)

- Podophages ("podo-" = foot) → Short tails (e.g., T7, P22).
- Myophages ("myo-" = muscle) → Contractile tails (e.g., T4, P1, Mu).
- Siphophages ("sipho-" = siphon/straw) → Long, flexible tails (e.g., Lambda, T1).

Microscopy and Structural Analysis

- High-resolution imaging (e.g., cryogenic TEM) helps study internal virion structures.
- Physical traits like symmetry, molecular architecture, and genome size can be inferred.
- Tail presence/type suggests entry mechanisms but does not determine host specificity or infection cycle.
- Filamentous phages' infection cycles can be inferred from morphology, but tailed phages require additional studies.

Historically → Phages classified by virion morphology (e.g., Podoviridae, Myoviridae, Siphoviridae).

Since 2022 → ICTV (International Committee on Taxonomy of Viruses) transitioned to sequence-based classification.

Current system (as of 2024) relies on:

- Phage genome sequencing, assembly, and annotation.
- Nucleotide comparisons & phylogenetic analysis to determine evolutionary relationships.
- Genomic-Based Classification & Reorganization-
- Example: Lambdavirus genus
- Previously classified by 15-70% nucleotide identity.
- Now members must share at least 70% nucleotide identity.
- Former members reclassified into separate genera.
- Example: Escherichia coli jumbo phage Goslar
- Originally under Myoviridae due to morphology.

- Later moved to Chimalliviridae based on protein similarities with PhiKZvirus (a Pseudomonas phage)

Phage Classification into Supergroups

- Broader classification beyond ICTV taxonomy
- group phages with shared functional/genomic traits

Supergroup Criteria

- Phages without high nucleotide similarity may still share:
- Genome type, size, and synteny (gene order & regulatory units).
- Core genes with related functions.
- Virion morphology or host cell type.
- Shared characteristic gene or gene set.
- Regulatory pathways and syntenic genomic organization.
- Protein-level similarities (amino acid sequences, conserved domains)

Examples of Well-Known Supergroups

Lambda-like Supergroup (Lambdoid phages)

- Characterized by mosaic genomes.
- Share four co-transcribed operons (late, left early, right early, immunity).
- Includes both temperate & lytic phages (e.g., lambda, P22).
- Found in E. coli, Salmonella and other hosts.

T4-like Supergroup

- Lytic myophages with 170 kb genomes.
- Infect diverse bacteria: Escherichia, Vibrio, Acinetobacter, Cyanobacteria, etc.
- Share ~30 core genes (e.g., DNA replication, synthesis, capsid proteins).

https//wwwtandfonlinecom/doi/epdf/102217/fmb1347?needAccess=true
https//wwwmdpicom/2079-6382/9/6/291
https//pmcncbinlmnihgov/articles/PMC10124553/#~text=Phages%20may%20also%20be%20used,even%20to%20combat%20global%20warming
https//wwwfrontiersinorg/journals/microbiology/articles/103389/fmicb201601152/full

https//journalsasmorg/doi/abs/101128/jcm225828-8341985

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Feb 11 2025

- **How does identifying the stability of phages in various environments help overcome the obstacles in implementing phage therapy-related techniques?**
- One of the obstacles in phage therapy is its stbaility in different environments
- Storage and transportation of the therapy has alos been a problem in it's approval and implementation
- Being able to identify the current stability phages have in different environments helps us identify
    - Current optimal environments we know phages thrive at
    - Pain points in phage stability that need to be improved upon for FDA-approval and easier implementation
    - Specific environmental factors that need to be taken into consideration to improve stability for when modifying the phages (either through evolution or gene editing)
- **What are the current optimal environments for phages to thrive?**
- Significant biological activity at pH levels 5-7
- No phage plaques are generally detected at pH level 3, while there is significant decline at pH 13
- Relatively stable at temperatures 20-50℃, with 37-40℃ being the general culturing, prep, and storage temperature, which is optimal for phages to grow and thrive before testing
- Significant titer reduction occurs at 60℃
- No plaques are generally observed at 70℃
- Various temperatures/pH levels experimented with include:
    - 20C
    - 30C
    - 40C

        - 50C
        - 60C
        - 70C
    - 1 hr in each pH environment used NaOH or HCl
        - 3
        - 5
        - 7
        - 9
        - 11
        - 13
- **The different applications and research areas in phage therapy?**
- Various applications include use in
    - Medical fields
    - Veterinary medicine
    - Agriculture
    - Sanitation/sterilization and overall clean and disinfecting
- Research has been done a lot in the medical field around more specific topics like
    - Combating AMR
    - Using it as a treatment option for bacterial infection developing antibiotic resistance
    - Drug/genetic material delivery carriers to specific target regions (not limited to just bacterial infections, gene editing RBPs in phages may make this possible) for more precise/personalized medicine
    - Using phages as tools to identify/diagnose different target issues, to confirm presence for less medical errors
    - Curing cancer/in cancer research

Feb 13 2025

- Found article to support our hypothesis
- https://www.epa.gov/goldkingmine/what-ph
- https://www.ncbi.nlm.nih.gov/books/NBK507807/#:~:text=In%20the%20absence%20of%20pathological,most%20important%20being%20blood%20oxygenation.
- https://pmc.ncbi.nlm.nih.gov/articles/PMC8006063/
- https://www.sciencedirect.com/science/article/abs/pii/S0927776517305805

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Feb 14 2025

Examples to support our applications

- https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(21)00612-5/abstract
- https://www.microbiologyresearch.org/content/journal/jmm/10.1099/jmm.0.001895
- https://clinicaltrials.gov/study/NCT04191148
- https://www.mdpi.com/1424-8247/14/11/1157
- Phage Therapy: Considerations and Challenges for Development
- https://journals.asm.org/doi/full/10.1128/cmr.00062-22
- https://pmc.ncbi.nlm.nih.gov/articles/PMC90351/

The 1960s Georgia Study (Soviet Union)

- Conducted in 1960s(a lil old so idk if we should add)
- preventative use of phages in over 30,000 children
- Study found dysentery rates in children treated with phages were up to 90% lower than people who received placebo

study by Slopek et al. (2000s)

- Used 550 patients with bacterial septicemia
- 92% success rate with phage therapy compared to antibiotics
- Because of Antimicrobial resistance (AMR)

Feb 28 2025

Set up lab procedure - Prepared bacteria culture by setting up 39 test tubes, and ensured consistent concentration of E. coli using LB broth.

Added more information to applications
https://www.mdpi.com/2079-6382/7/4/95
https://pmc.ncbi.nlm.nih.gov/articles/PMC1891379
https://www.nature.com/articles
https://www.cdc.gov/antimicrobial-resistance/media/pdfs/pseudomonas-aeruginosa-508.pdf
https://aricjournal.biomedcentral.com/articles/10.1186/s13756-023-01355-4
https://pmc.ncbi.nlm.nih.gov/articles/PMC11321567
example on how phage is viable alternative to AMR

Pseudomonas aeruginosa - common infection you can get at hospital which developed resistance to multiple antibiotics.

- a systematic review and meta-analysis showed that resistance rates in Pseudomonas aeruginosa ranged from 20.9% for amikacin to 98.72% for ceftriaxone
- study found that 23.4% of P. aeruginosa isolates were resistant to both meropenem and ciprofloxacin
- some multidrug-resistant strains are resistant to almost all antibiotics, including carbapenems
- phage therapy as an alternative treatment.
- Research has shown efficacy of phage therapy against P. aeruginosa infections. ex: study showed that administering a P. aeruginosa-specific phage cocktail significantly reduced mortality in thermally injured, P. aeruginosa-infected mice
- Clinical settings, personalized phage therapy has led to significant improvements in patients with chronic lung infections caused by P. aeruginosa, even when complete bacterial eradication was not achieved

Started to format research to add to trifold/ CYF website
Added extra info with these links - https://pmc.ncbi.nlm.nih.gov/articles/PMC4418462/

Bacteriophages, more commonly known as phages, are viruses that only infect a single bacterial species or a specific strains within a species. Phages are composed of a "head" with nucleic acid genome in a capsid protein shell, a "tail", and "legs". Phages are non motile(unable to move on their own), this means that they rely on brownian motion(random movement caused by particles colliding with one another).

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Antibiotic-Related Educational Activity in Lao PDR. *Antibiotics*, *7*(4), 95. https://doi.org/10.3390/antibiotics7040095

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Shbaita, S. (2023, December 20). Antibiotic resistance profiles and associated factors of Pseudomonas Infections among patients admitted to large tertiary care hospital from a developing country. *Antimicrobial Resistance & Infection Control*, *12*(149). https://aricjournal.biomedcentral.com/articles/10.1186/s13756-023-01355-4

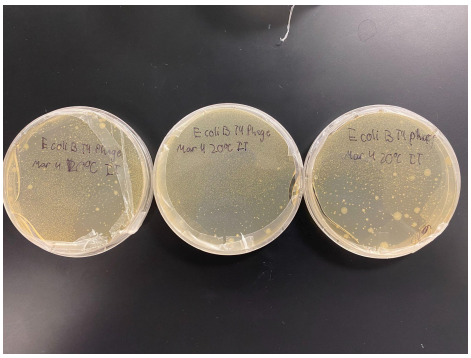
Mar 4 2025

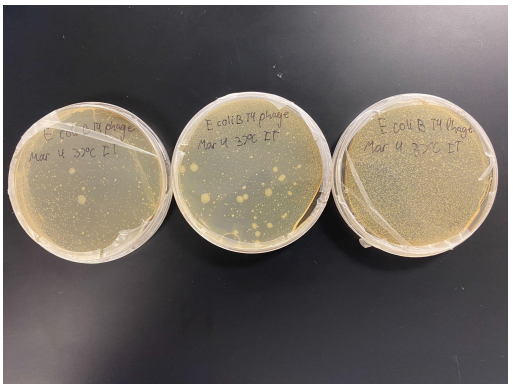
- Today went into the lab to begin our phage stability tests
    - Made sure the OD600 of all the phages were between the levels 0.08-0.12
    - Had to cut down our experiments to the following
        - pH tests
            - 5
            - 6
            - 7
            - 8
            - 9
        - Temperature tests
            - 20℃
            - 37℃
            - 60℃
    - Plated the phages
    - Some of the agar wasn't fully smooth so that may impact growth and demonstration of results

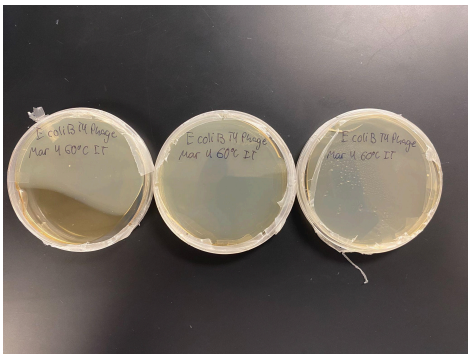
Mar 6 2025

- Went into the lab to see our results

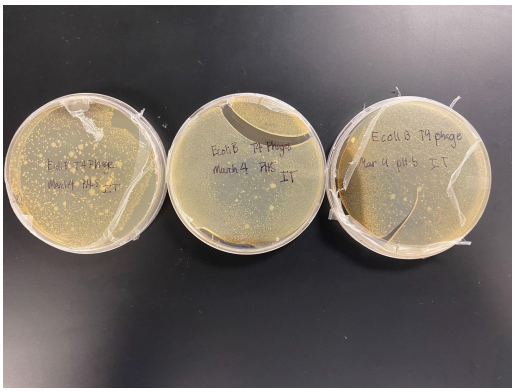
- The plates did not show anything in relation to the phage performance but only for bacterial colonies
- Procedure was not 100% correct to be able to get the results we needed, so we are scheduling another time to re-perform the experiment
- Results from the tests

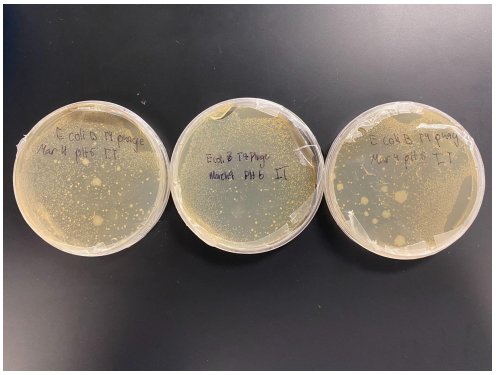




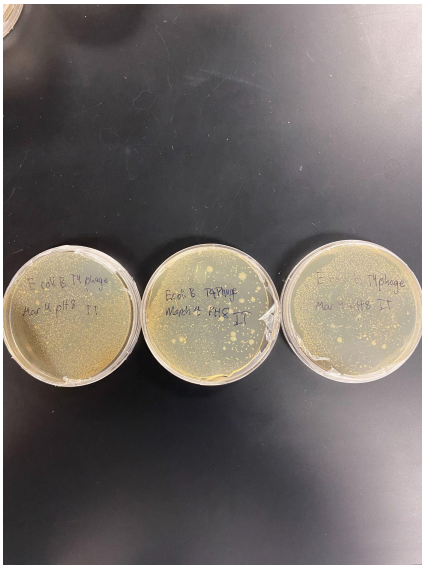


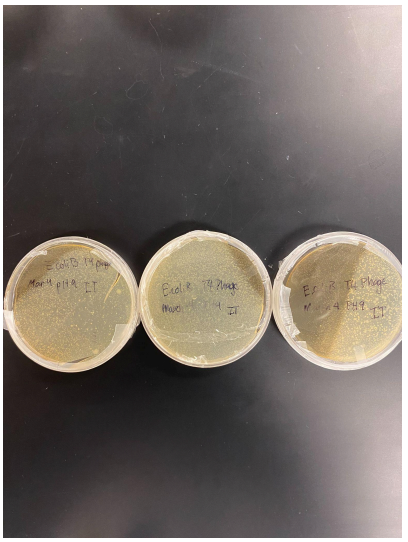
Temperature





pH





- Modifications
    - Need to treat phages separately and expose them to the different conditions
    - Plate the bacteria on the petri dishes first separately
    - Add the treated phages on top
    - Then be able to compare
    - Make sure bacteria is on a flat surface

Research to aid in refining procedure/research
How to obtain a liquid sample of phage from a plaque (or putative plaque) on a lawn of bacteria.
https://pmc.ncbi.nlm.nih.gov/articles/PMC9323186/
https://pmc.ncbi.nlm.nih.gov/articles/PMC7149772/
https://academic.oup.com/ve/article/10/1/veae028/7642516

Formatting research section for our online project log

What are Bacteriophages?

Bacteriophages, also known as phages, are viruses that only infect a single bacterial species or specific strains within a species. They are composed of a "head" with a nucleic acid genome in a capsid protein shell. For our experiment our goal was to test how effective phages are under different pH and temperature conditions.

Phages are classified by their structure and replication cycle and taxonomy. There are three different types of ways that phages asexually reproduce:

Lytic Cycle (Virulent Phages): Phages that infect a host cell to hijack the host's machinery, to propagate. The host cell then is lysed (broken open), releasing new phages. Clear plaques will show up when lytic cycle phages are cultured on bacterial lawns, because the infected bacteria are destroyed.

Lysogenic Cycle (Temperate Phages): When phages inject their genome, this is called prosphage. Prophage will either be dormants as a circular episome that replicates circular dna independently or be integrated into the bacterial chromosome, in this case the host's dna will be inherited by all of the daughter cells (8 -10). to conditions like stress, the prophage might switch to the lytic cycle,which would start phage replication killing the host cell. Lysogenic phages like Lambda phage usually form turbid plaques (cloudy areas) because they do not kill their host cells immediately.

Chronic Infection Cycle (Filamentous Phages): Phages that continuously replicate without causing host lysis. The host cell produces new phage particles that are secreted, but the host cell remains intact. Filamentous phages, such as M13 phage replicate by secreting virions, instead of killing the host phage particles are excreted through the cell membrane.

This makes them hard to differentiate at times from lysogenic phages but their replication does not have a dormant prophage stage.

Another characteristic phages are classified based is their morphology, this would include traits like shape, tail type, and size.

Non - tailed phages: Just as the name suggests these phages do not have tails. The three known examples of these types of phages are filamentous phages (e.g., M13),pleomorphic phages (e.g., phi6), and polyhedral. These phages usually have an elongated or variable shape and typically have simpler mechanisms for attaching to and infecting host cells.

Tailed Phages (Caudovirales Order): Have a tail to control the injection of phage genomes into their host. There are 3 subclasses to this type of phage:

Podophages: Have short tails (e.g., T7 and P22). They inject DNA by attaching the tail fibers to the surface of the host, then administering their genome.

Myophages: This is the category that T4 phages fall into, these phages have a contractile tail, which allows them to inject its genome by contracting the tail sheath.

Siphophages: Have long flexible tails that help to attach themselves to bacterial cells, these phages like Lambda can not contract their tails like myophages.

Why we chose T4 Phages

What is Phage Therapy?

With the rise of antimicrobial resistance (AMR) phage therapy has become a viable option to work alongside antibiotics. Introduced in the 1900s phage therapy is using these viruses(phages) to infect a specific strain of bacteria.

Only lytic phages are used in phages therapy, as phage production in the bacteria causes disruptions in its metabolism, eventually killing the bacteria. They do this by encoding Holins, endolysins, and spanins to break the cell wall and cell membrane.

Different Applications, Advantages, and Obstacles in Implementing Phage Therapy

Various applications of phage therapy include

- Medical fields
- Veterinary medicine
- Agriculture
- Gene editing

A lot of the research of phage therapy applications has been tied to the medical fields. Currently, there has been a development of the antibiotic resistance crisis, a problem that has occurred due to the overuse of antibiotics, causing bacteria to develop various levels of antibiotic resistance. Various bacterial strains have become superbugs, meaning they have

been identified by WHO as bacterial strains that have complete immunity to all antibiotics, making them lethal. Phage therapy is considered to be the final solution that needs to be developed in order to combat antibiotic resistance in bacteria.

As research has been done mainly in the medical fields, there has been more development of research for phage therapy in those other fields listed above. Some of the other specific medical applications of phage therapy, besides combating antibiotic resistance, include

- Using it as a treatment option for bacterial infection developing antibiotic resistance
- Drug/genetic material delivery carriers to specific target regions (not limited to just bacterial infections; gene editing RBPs in phages may make this possible) for more precise/personalized medicine
- Using phages as tools to identify/diagnose different target issues, to confirm presence for less medical errors
- Curing cancer/in cancer research

There are many advantages to exploring and developing phage therapy, which make it a unique treatment that, if obstacles in its implementation can be combated, can be a very successful treatment.

Phages are very specific as to what they can infect. For example, one phage may only be able to infect an E. coli bacterial strain, while another phage may only be able to infect a staphylococcus bacterial strain. Because phages are so specific as to what they can infect, it makes it much easier to guarantee that they won't mess with other body systems or cause multiple side effects. It was actually reported in some of the earlier tests that patients experienced around little to no side effects with the use of phage therapy because of how precise and selective they are.

Phages are living organisms that depend on infecting bacteria in order to continue to thrive and reproduce. That means, as bacteria continue to develop and evolve to protect themselves against phages, phages will also continue to evolve to overcome the obstacles for infection that bacteria may create through the generations. The key difference between phages and antibiotics is that phages can continue to evolve to keep up with bacteria, while antibiotics are only as good as we can make them, inevitably to a point where bacteria will become stronger and immune, like the problem we are currently facing.

Phages are easily customizable for different purposes like I mentioned above, like drug delivery, modification of phage target, and much more. They are easy to be implemented into personalized therapy, which is the more advanced healthcare experts are researching and looking to implement more and more.

There are a few obstacles that need to be overcome in order for phage therapy to be FDA-approved.

Phages are very specific as to what they can infect, which was mentioned to be an advantage. But, this characteristic also causes an obstacle. If we are to become partially or fully dependent on phage therapy, we need to make sure we can get access to various species of phages and create a phage library that holds phages that can infect multiple different types of bacteria. Or, we would need to develop technology like gene editing to modify the RBPs of phages so we can control what kind of bacteria the phage targets when we need it to.

Phages are living organisms that thrive best in specific environmental conditions. In order for phage therapy to be FDA-approved, we need to ensure that phages can successfully infect bacteria in various environmental conditions ranging in temperatures and pH (mainly pH if

specifically for human applications). We also need to make sure that the phages can be long-lasting until use and can survive in various storage conditions that will range in both temperature and pH; that way, they won't expire quickly, wasting both time and resources to create more of these treatments until use.

Another obstacle in phage therapy implementation is consistency. We need to make sure that the phages will be consistently safe to administer to the body through the process of more large-scale testing/clinical trials. We also need to ensure that phages will have about the same success rate in killing bacteria every time they are deployed. In order to become partially or fully dependent on phage therapy in the future, we need to be sure that they can consistently work.

An obstacle in phage therapy implementation is its interaction with the immune system. One of the concerns is that phages, once administered to the body, will be mistaken by the immune system as a threat. While this hasn't always happened, we need to find ways to mask the phages or trick the immune system into not interacting with the phages as they are looking for the bacterial target.

One last obstacle is the biofilms that some bacteria have as a sticky protective layer. The biofilm can prevent the phage from being able to bind and access the bacteria to infect it, and there has been research on gene editing phages to overcome this obstacle as well.

Current Optimal Environments for Phages to Thrive

Phages are somewhat able to thrive in various environmental conditions. They have enough adaptability to survive in different temperatures and pH levels, but we still need to determine how well they will be able to perform in targeting and infecting bacteria in those changes in conditions.

As for pH levels, research has shown that phages thrive at pH levels 5-7, with significant biological activity between those ranges (Cong, 2024). The pHs tested in this study were 3, 5, 7, 9, 11, and 13, to be able to test an acceptable range of acidity and basic. At the most basic side tested, pH 13, there was significant decline in phage titer. At the most acidic tested, pH 3, there were generally no phage plaques detected.

For temperature resistance, the phages thrived the best between temperatures 20℃ - 50℃ (Cong, 2024). There is significant titer reduction at 60℃ and generally complete titer loss at 70℃ (Kering, 2020). Between 37℃ - 40℃ are optimal temperatures for growing and culturing phages, especially before use. 4℃ is a good temperature for storing the phages for longer periods of time until use. In these two studies, the temperatures 50℃ - 70℃ were tested. In the study by Cong in 2024, the additional temperatures 20℃, 30℃, and 40℃ were tested. In the study by Kering in 2020, it was shown that exposing the phages to temperature treatment triggered adaptive evolution and made the mutant phages more resistant to the varying temperatures.

Importance of Identifying Phage Stability to Improve Phage Therapy

One of the current obstacles in phage therapy already identified is stability of phages both in different environments when being administered and when being stored for long periods of time until use. Phages need to be stored, able to be transported, and effective as a

treatment in various environmental conditions in order to be reliable and eligible to be FDA-approved. Being able to identify the current stability advantages and pain points for phages helps us identify

- Current optimal environments we know phages thrive at
- Pain points in phage stability that need to be improved upon for FDA approval and easier implementation
- Specific environmental factors that need to be taken into consideration to improve stability for when modifying the phages (either through evolution or gene editing)

This will allow us to further our research and target our resources in tackling the most prominent challenges.

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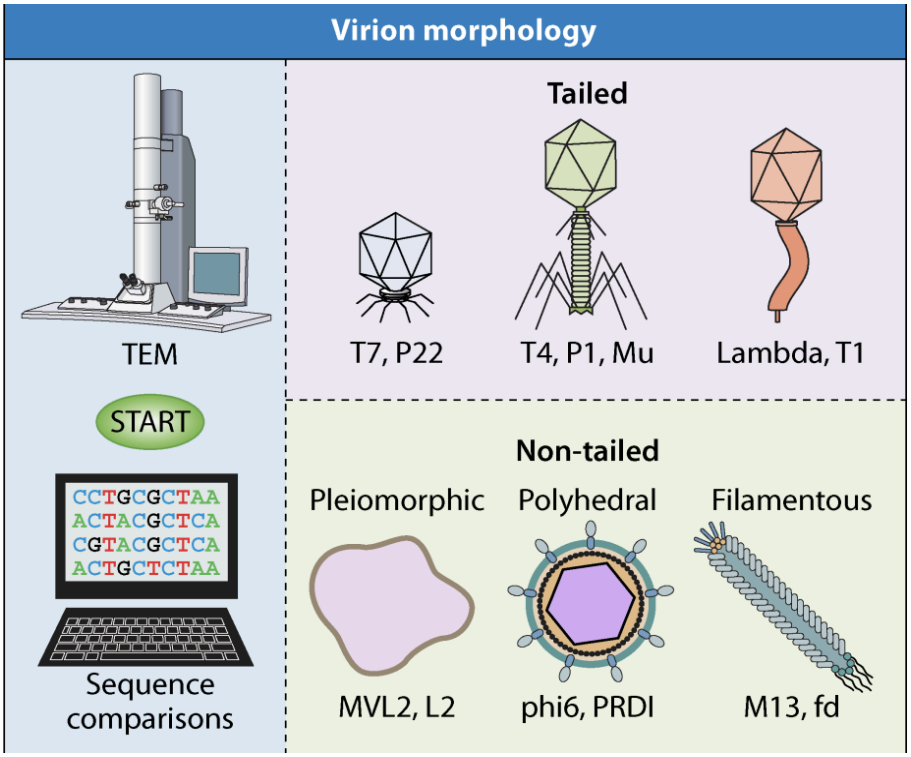
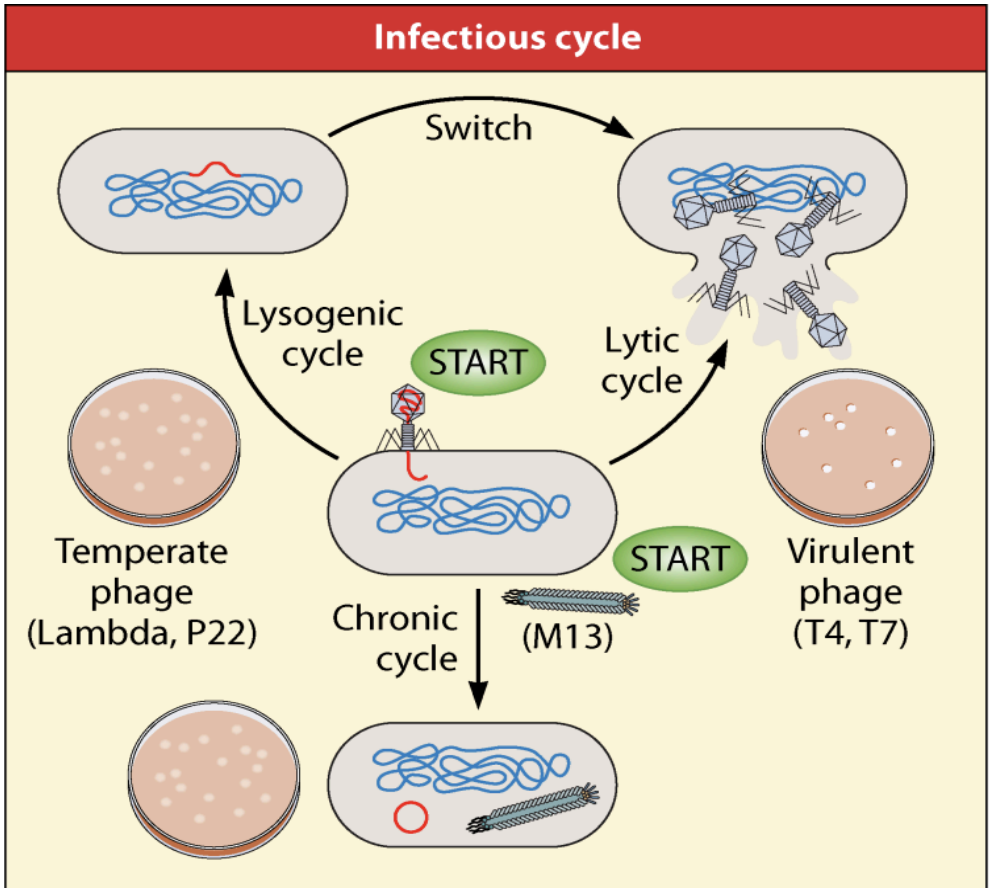
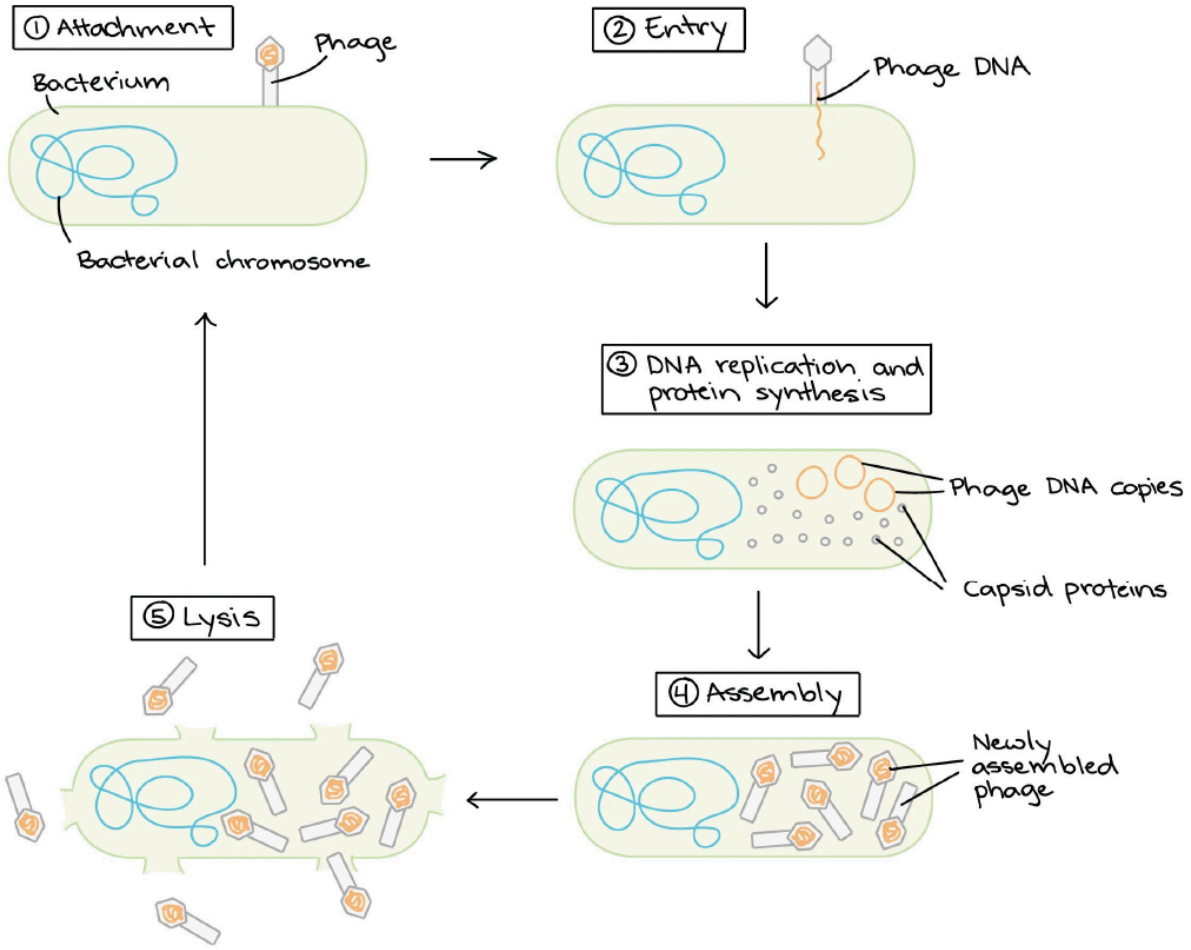
Mar 9 2025

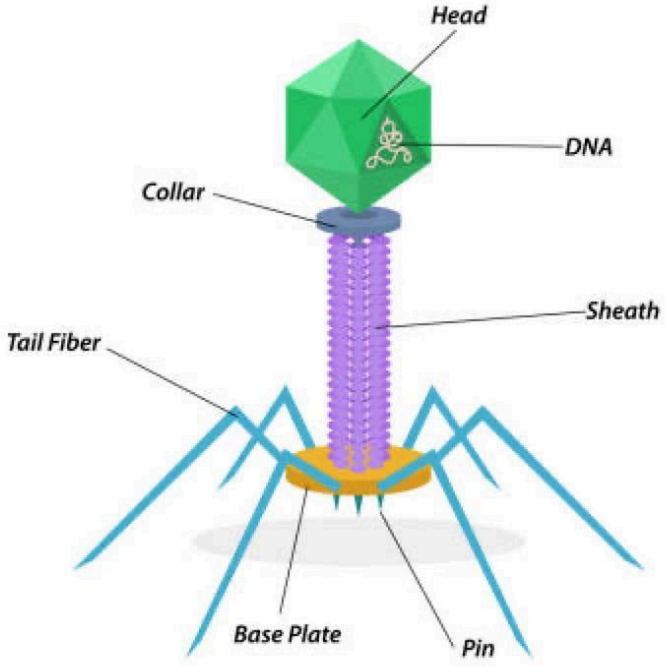
Found images for our presentation(Isa do you mind adding yours so we can decide what to put on our trifold)



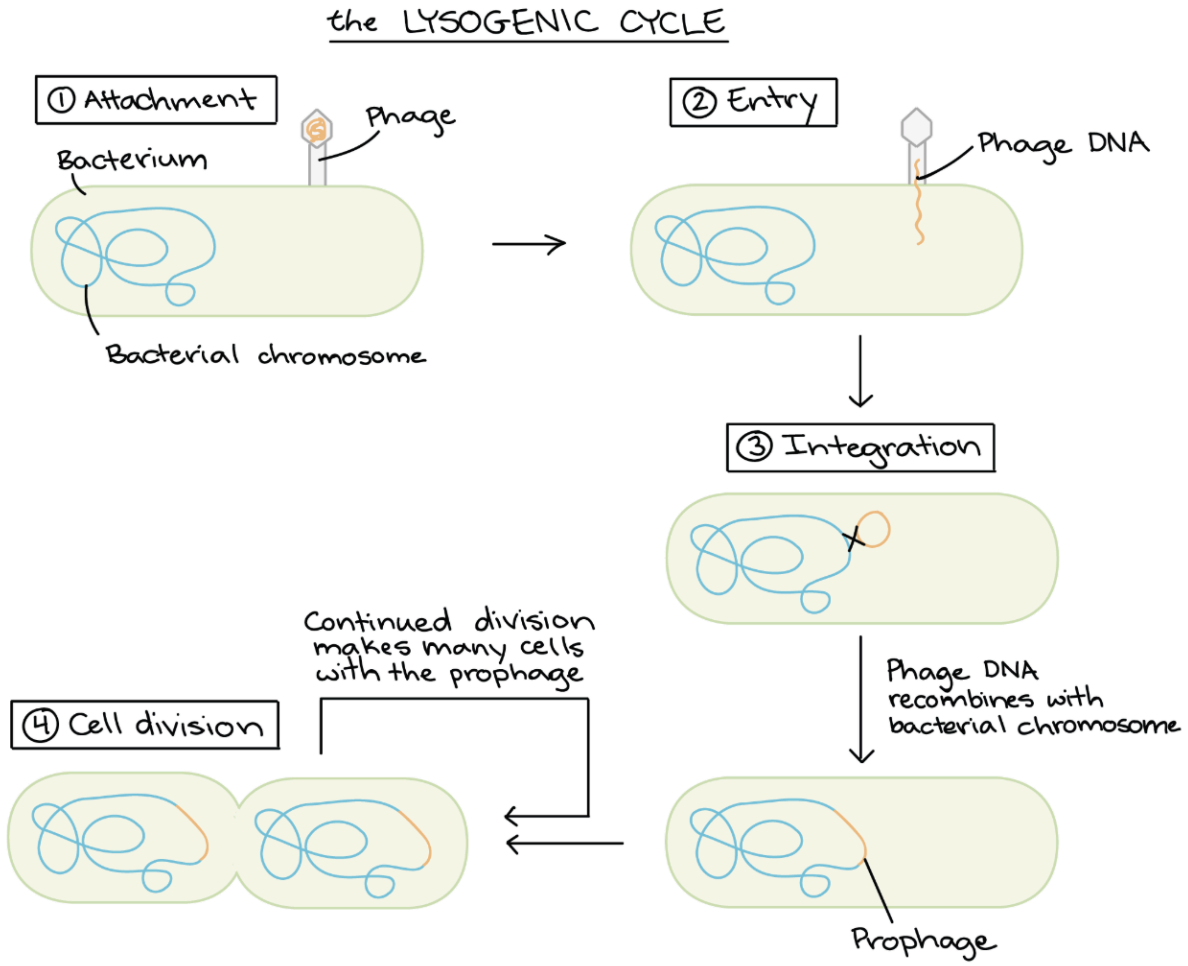


the LYTIC CYCLE





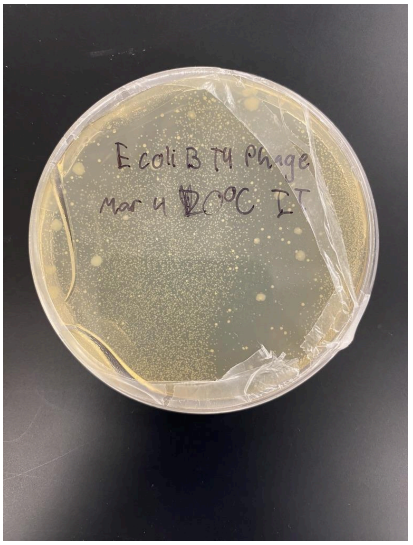
BACTERIOPHAGE STRUCTURE

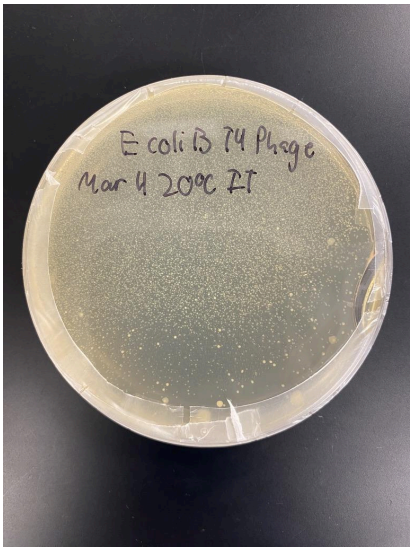


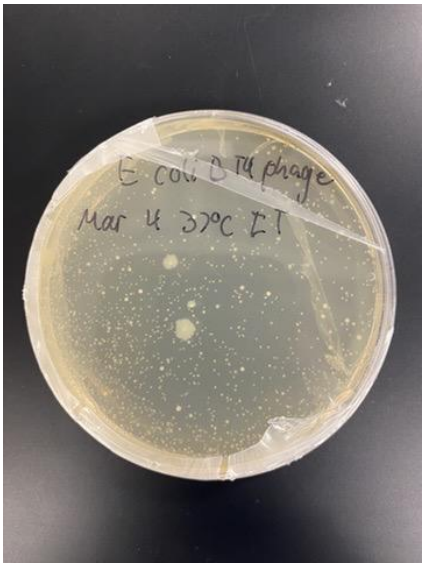
Mar 11 2025

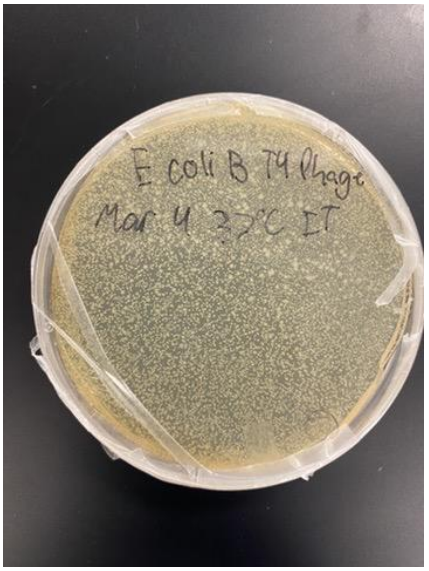
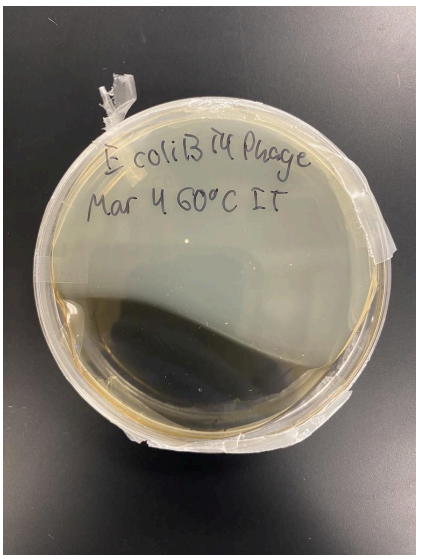
- Documenting our observations more clearly

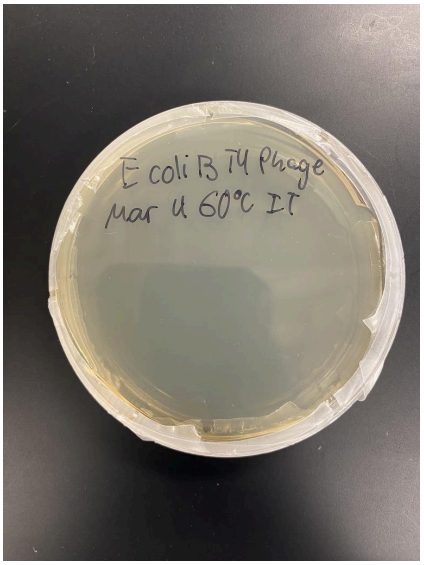
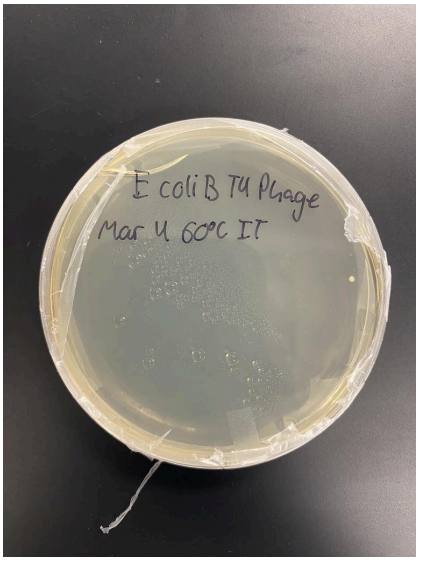
Temperature

Temperature	**Triplicate**	**Image**	**Observation**
20℃	#1		Not the results we are looking for. Bacteria come up in the yellow spots, noted as colonies. Not showing results of phage, although there was present infection. There are a few prominent bacterial colonies.

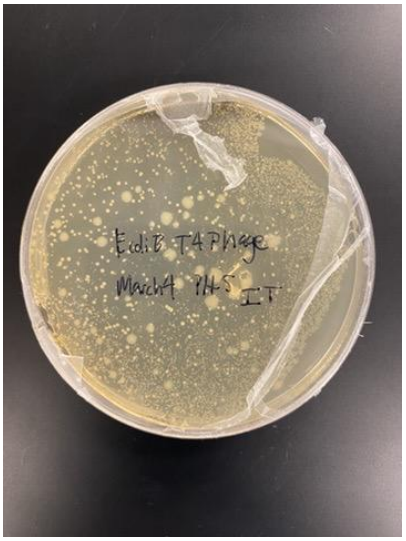
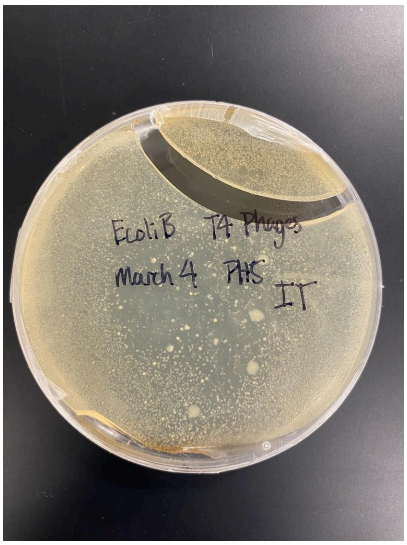
20℃	#2		Smaller scatter of bacterial colonies. Still no plaques being identified.
20℃	#3		Largest of the 20℃ triplicate of bacterial colonies. The plaques did not show up on this petri dish, not showing the infection areas.

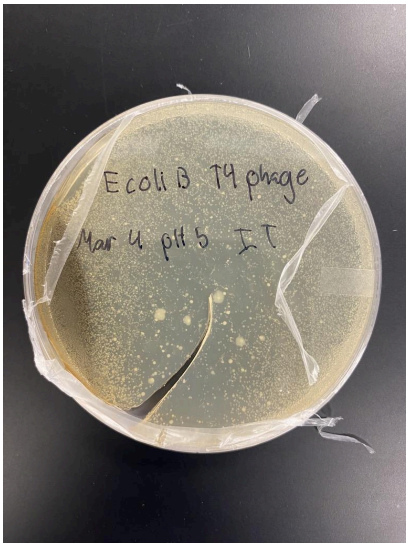
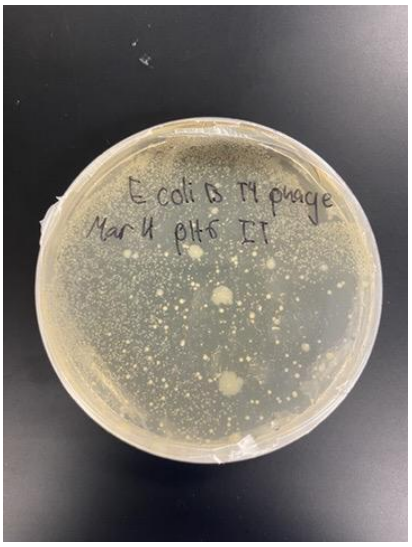
37℃	#1		Fair bacterial colony activity, around the same amount as in 20℃. Can guess that phages had better infection rates here.
37℃	#2		More bacterial colonies here. Not showing phage plaque formation.

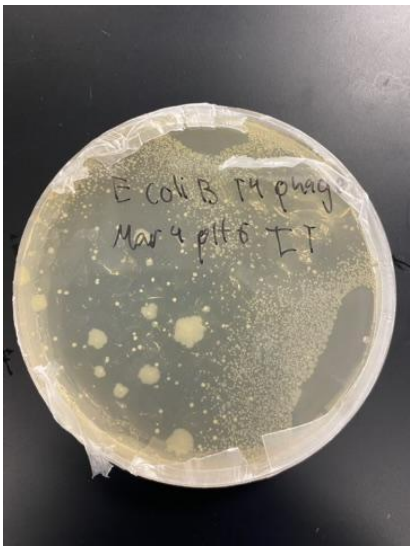
37℃	#3		Scatter of bacterial colonies, still no plaque formation.
60℃	#1		Very little, one or two bacterial colonies at sixty. Probably no phage or bacterial action at this temperature.

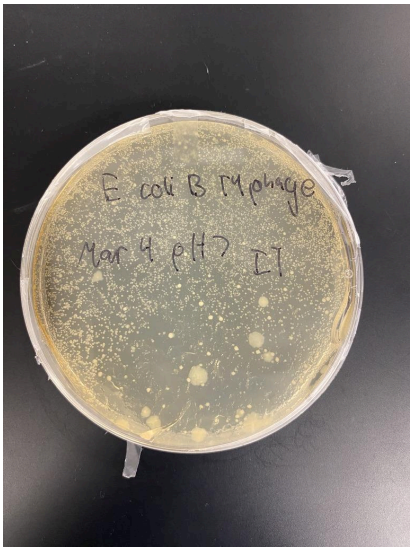
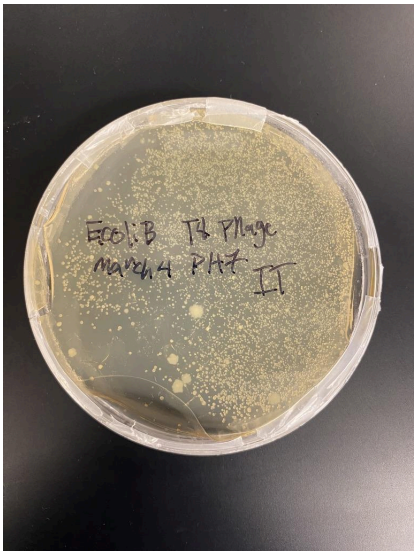
60℃	#2		No bacterial colonies or plaques.
60℃	#3		Moisture build-up. One or two bacterial colonies, no phage activity is detected.

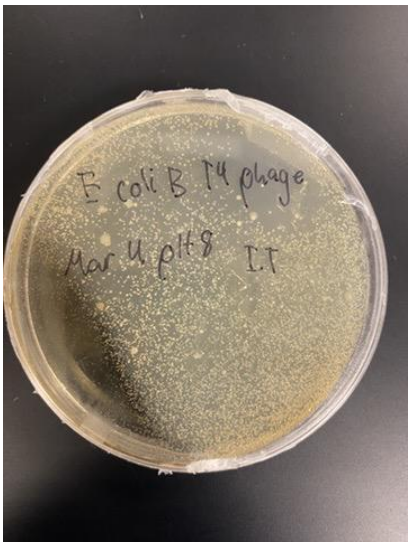
pH

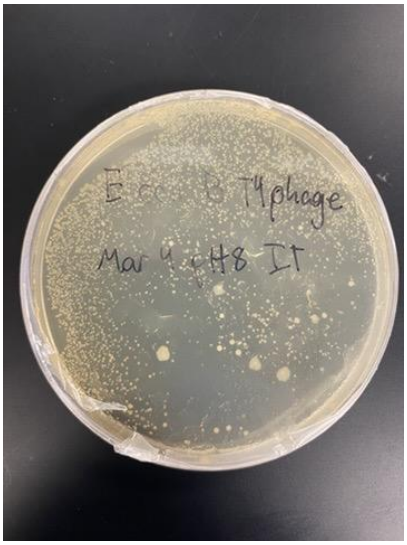
pH	Triplicate	Image	Observation
5	#1		Similar to temperature tests, there are no signs of phage plaques, but there are bacterial colonies. There are quite a few in this petri dish.
5	#2		Scatter of bacterial colonies, no phage plaques. Agar is broken up.

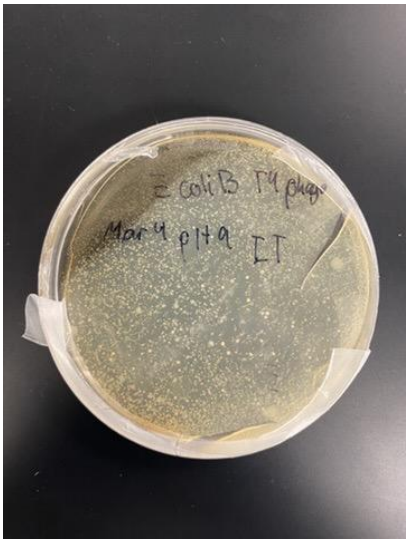
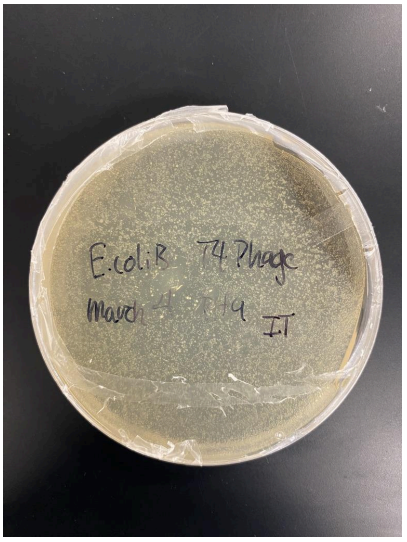
5	#3		Agar is broken a little. Scatter of bacterial colonies, no phage plaques.
6	#1		Cannot identify phage activity, fair amount of bacterial colonies.

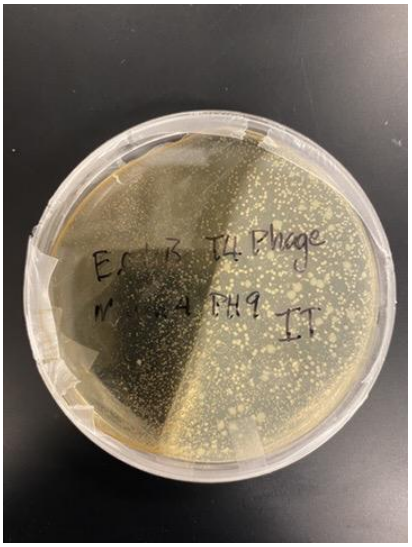
6	#2		Scatter of bacterial colonies, no plaques.
6	#3		Larger bacterial colonies, no phage plaques.

7	#1		Larger amount of bacterial colonies as well as fairly scattered, no plaques for phage activity.
7	#2		Scatter of bacterial colonies, no phage plaques.

7	#3		Scatter of bacterial colonies with a couple large colonies, no plaques.
8	#1		Scatter of bacterial colonies, no plaques.

8	#2		Scatter of bacterial colonies, no plaques for phage activity.
8	#3		Larger amounts of bacterial colonies, no plaques.

9	#1		Scatter of bacterial colonies, rather minimal. No plaques for phages.
9	#2		Minimal scatter of small bacterial colonies, no phage plaques.

9	#3		Agar somewhat broken, didn't settle correctly. No phage plaques. Scattered bacterial colonies.

- Emailed James La to see if we could confirm our new lab dates to repeat the experiment
- Finished formatting everything for the trifold, will print tomorrow
- Image links
- https://www.researchgate.net/figure/The-main-strategies-for-phage-therapy-The-black-circle-indicates-that-a-variety-of_fig2_344962336
- https://globalfoodsafetyresource.com/can-phage-therapy-replace-antibiotics-in-growth-promotion/
- https://www.vitares.org/en/magazine-en/143-phage-therapy-a-modern-approach-against-antibiotic-resistance
- https://www.labiotech.eu/in-depth/phage-therapy-antibiotic-resistance/

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- Writing out our analysis as well

After conducting our experiments and looking at the petri dishes, we couldn't see any plaques. Plaques are where the phages have performed lysis on the bacteria. We were going to count out the number of plaques we saw on the petri dish, then use those numbers to calculate the rate of infection and the overall phage effectiveness infecting bacteria in various conditions.

All we could observe on the petri dishes were isolated bacterial colonies, which shows that there was growth of culture. We weren't able to observe any phage activity in the form of plaques. In our experiment, we mixed the phages and bacteria together and exposed them to the specific environmental condition for 30 minutes to allow the phage to attempt to infect the bacteria. Then, we would plate the phage/bacterial mixture and be able to view the results. Our thought process behind this was to simulate the phage finding a bacteria in the specific environment and then having to infect it.

After finishing our experiment, we found that this part of our procedure was flawed. What we learned was that we should have taken the phages and exposed them to the specific environmental condition separate from the bacteria because this experiment is focusing on phage efficacy, not bacteria efficacy. Then, after exposing that phage to the condition for the desired duration, 30 minutes for our experiment, we would then add it to the bacteria straight on a petri dish. The bacteria would be cultured separately as we had already done, but then we would plate the bacteria to form a bacterial lawn on the petri dish. Then, we would take

our treated phages and streak it over that lawn with an inoculation loop. Then, we would incubate and allow the treated phages to infect and see how well they could infect after being treated with a specific condition.

The flaw is that we wouldn't be able to see the results of the phages infecting the bacteria in the procedure that we used. But there was still infection and activity of the phages, which wasn't the issue.

What we can take from this is to see the growth of the bacteria after being exposed to the varying environments. Also, since there was around equal concentration of bacteria, we can see from the varying colonies that were produced either

- Where phages were more/less active
- Where the conditions were more/less optimal for growth
- Where bacteria were able to survive the phages

It is hard to determine which result is which, so we can't make any definite conclusions from our experiment.

- Wrote out conclusion

Because our experiment had a flaw in showcasing our results, we cannot come to any definite conclusion about our hypotheses. We can confirm that there was bacterial growth, and we can infer that there must have been phage lysis, even though it couldn't have been reflected in plaques on the petri dishes due to the procedure flaw. The bacterial colonies that were shown in the petri dishes could show us the relative optimal/non-optimal conditions for phages to thrive and eliminate bacteria based on the number of colonies. But, we can't be for sure what each number of colonies on the petri dishes represent. For example, if nothing appeared on a petri dish, it could either be because everything perished, meaning the condition wasn't optimal, or that the phages performed lysis on all the bacteria, meaning the condition was still optimal. But we wouldn't be able to tell without those plaques being shown. Our next step is to return to the lab to repeat our experiment and adjust our procedure so that we can gather data and find conclusions to our hypotheses.

- Site: https://journals.plos.org/plosone/article/file?id=10.1371/journal.pone.0276603&type=printable

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Li, Y. (2020, October). *Phage Therapy: Consider the Past, Embrace the Future* [Graphic]. ResearchGate. Retrieved March 11, 2025, from https://www.researchgate.net/figure/The-main-strategies-for-phage-therapy-The-black-circle-indicates-that-a-variety-of_fig2_344962336

Osborne, S. (n.d.). *Can Phage Therapy Replace Antibiotics in Growth Promotion?* Global Food Safety Resource. Retrieved March 11, 2025, from https://globalfoodsafetyresource.com/can-phage-therapy-replace-antibiotics-in-growth-promotion/

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Mar 12 2025

- Prepared trifold for school-wide science fair tomorrow
- Presented data and formatted it for trifold

Mar 13 2025

- Presented trifold to school-wide science fair
- Confirmed new dates to return to lab

Mar 18 2025

- Went into lab for second time with new supervisor
- Have new procedure
- Created ph buffers for testing
    - 3
    - 7
    - 11
- Prepared phages and bacteria for testing

New procedure:

Day 1 - Overnight culture of *E. coli*

Date: March 17-18, 2025

Objective

- Grow an overnight culture of *E. coli*
    - Three were overnights were grown although only two is required for the experiment

Methods

Overnight culture of E. coli

1. Add 5 mL of LB broth in a test tube.
2. Inoculate a single colony of *E. coli* into the test tube using an inoculation loop.
3. Incubate *E. coli* culture overnight (~18-22 hrs, no more than 24 hours) at 37°C with shaking (250 rpm).

Notes

- Three overnight cultures of *E. coli* were prepared and placed in the incubator at 37°C with 250 rpm at 3:57 PM (March 17, 2025)
    - Overnight cultures were removed from the incubator at 1:10 PM (March 18, 2025)

- Overnight #1 — 0.154 OD_{600}
- Overnight #2 — 0.284 OD_{600}
- Overnight #3 — 0.156 OD_{600}

Day 2 - Infection and Propagation of T4 Bacteriophage

Date: March 18, 2025

Objective

- Infect an overnight culture of *E. coli* with T4 bacteriophage for amplification

Methods

Bacteriophage Infection and Propagation (Amplification)

1. Infect 5 mL of the overnight culture with 100 µL of T4 bacteriophage stock solution
2. Incubate phage-infected *E. coli* culture at 37°C and 250 rpm (shaking) for 3-4 hours (bacterial culture should become clear).
3. Centrifuge the phage-infected *E. coli* culture at 10,000 x g for 10 minutes.
4. Collect the lysate/supernatant (solution above the bacterial pellet).
5. Filter the lysate/supernatant through a 0.22 µm filter using a syringe.
6. Store filtered lysate/supernatant at 4°C.

Notes

- *E. coli* was infected with T4 bacteriophage and placed in the incubator at 1:21 PM (March 18, 2025)

Day 3 - Plaque Forming Assay of T4 Bacteriophage against *E. coli*

Date: March 19-20, 2025

Objective

- Perform plaque forming assay with T4 bacteriophage against *E. coli*
- Determine PFU/mL of phage lysate

Methods

Plaque Forming Assay

1. Melt LB soft agar and keep heated at 65°C
2. In a separate Eppendorf tube, prepare 100 µL of a bacterial inoculum adjusted to 0.1 OD_{600} with LB broth.
3. Transfer 50 µ*L* of 0.1 OD_{600} the *E. coli* suspension into 5 mL of Molten Soft Agar.
4. Add 100 µL of T4 lysate
5. Pour onto hard agar plate and swirl
6. Wait until soft agar has solidified
7. Parafilm plates and incubate for 18-24 hours at 37°C

Determining PFU/mL

1. Count plaques on plates and calculate the PFU/mL (formula below):

$$PFU/mL = \frac{\#\ of\ plaque\ counts \times dilution\ factor}{volume\ of\ inoculum}$$

   - **# of plaque counts** = number of plaques (clear zones) on plates
   - **Dilution factor** = factor by which phage was diluted before plating
   - **Volume of inoculum** = volume of phage plates in mL

Day 4 - pH and Temperature Stability of T4 Bacteriophage

Date: March 19-20, 2025

Objective

- Subject T4 bacteriophage to various pH and temperature conditions
- Determine PFU/mL of phage lysate

Methods

Preparing pH-treated T4 Phage

1. In a separate eppendorf, adjust phage stock to ~10^7 PFU/mL.
2. In three separate eppendorf tubes, aliquot 100 µL of T4 phage solution into each eppendorf
3. Add 100 µL of pH-adjusted buffer solution to respective eppendorf tubes
4. Incubate at 37°C for 60 minutes
5. Perform again for each pH treatment (pH untreated (LB broth), 3, 7, and 11) and for temperature (room temperature, 4°C, 37°C, 60°C)
    a. Temperature
        i. 4°C — Fridge
        ii. 20°C — Room temperature (or incubator)
        iii. 37°C — Incubator
        iv. 60°C — Heat block or Hot water bath

Plaque Forming Assay

1. Prepare 100 µL of *E. coli* at 0.1 OD_{600} from overnight culture
2. Pipette 50 µL of this bacterial liquid culture into 5 mL of molten soft agar

3. Add 100 µL of pH-treated T4 phage lysate
4. Pour onto hard agar plate and swirl
5. Wait until soft agar has solidified
6. Parafilm plates and incubate for 18-24 hours at 37°C

Determining PFU/mL

1. See Day 3 for steps.

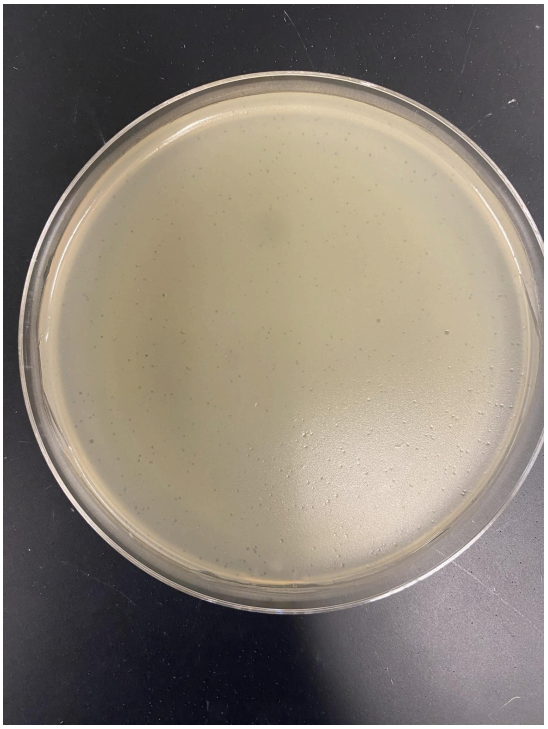
- Updated our online log of our project on the platform
- Began writing project report

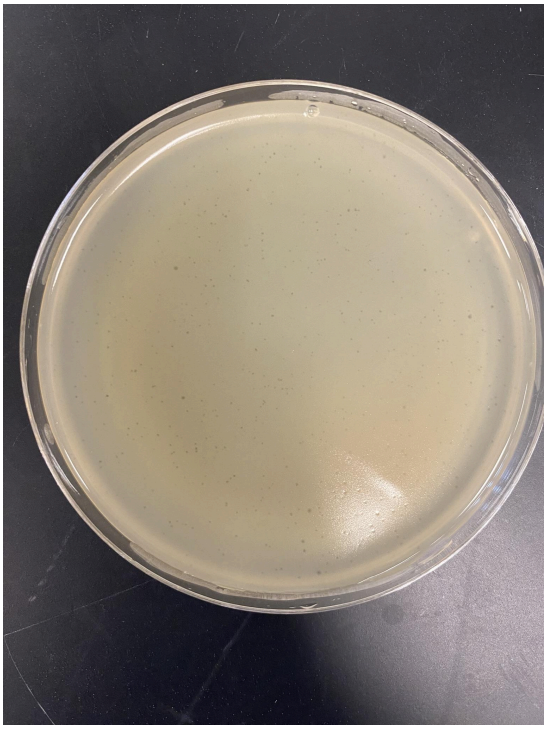
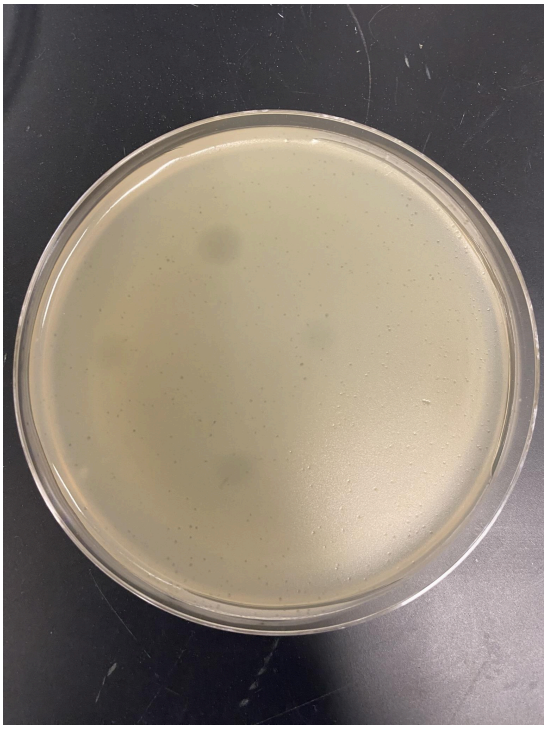
Mar 19 2025

- Went into the lab to perform day 2 of the protocol
- Diluted and cultured three phages plates to get an average concentration of the phage

Mar 20 2025

- Went in for the day 3 of the protocol
- Gathered data to create average phage concentration level
- Treated the phages by exposing them for 1 hour at each condition
- Plated them with the bacteria, being cultured overnight for results tomorrow

Plate	Photo	Notes
1		Number of plaques: 532

2		Number of plaques: 580
3		Number of plaques: 408

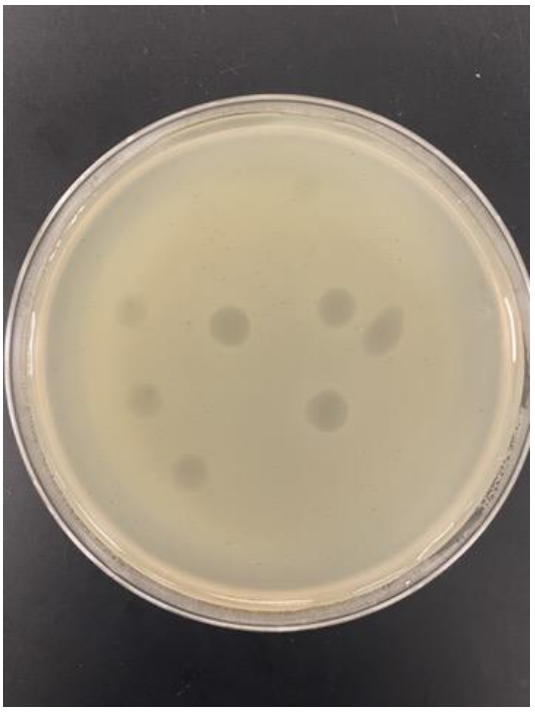
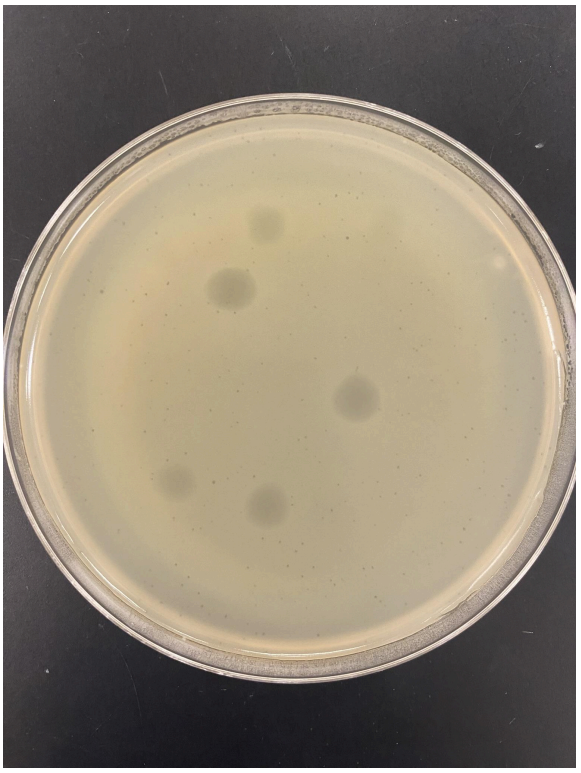
- Used formula to calculate PFU/mL:

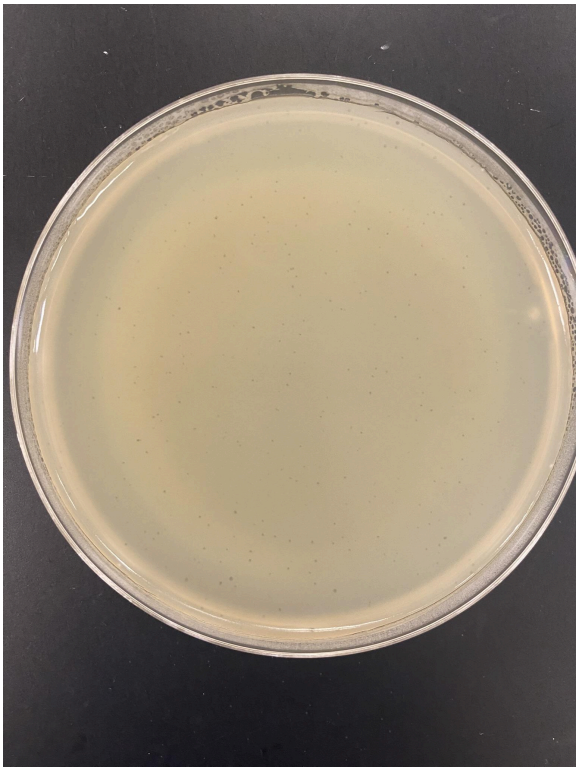
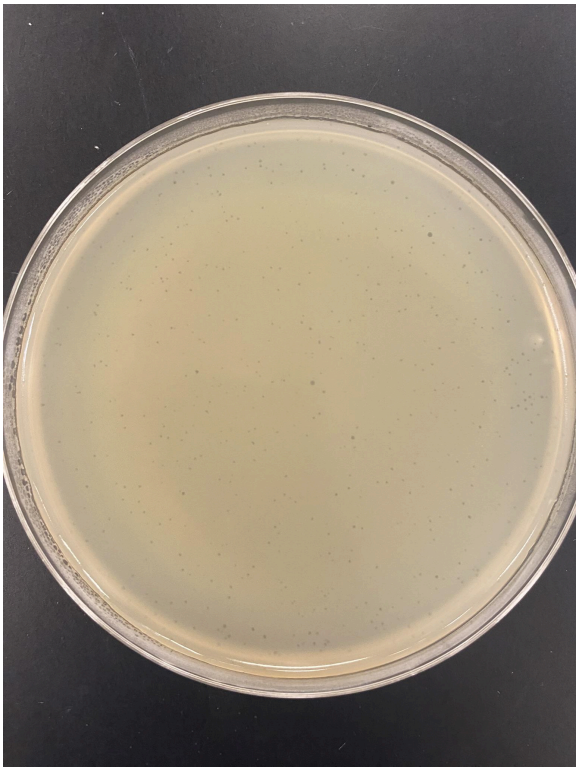
532 (number of plaques) x 100 (dilution factor)/0.1mL (amount of phages used) = 532000

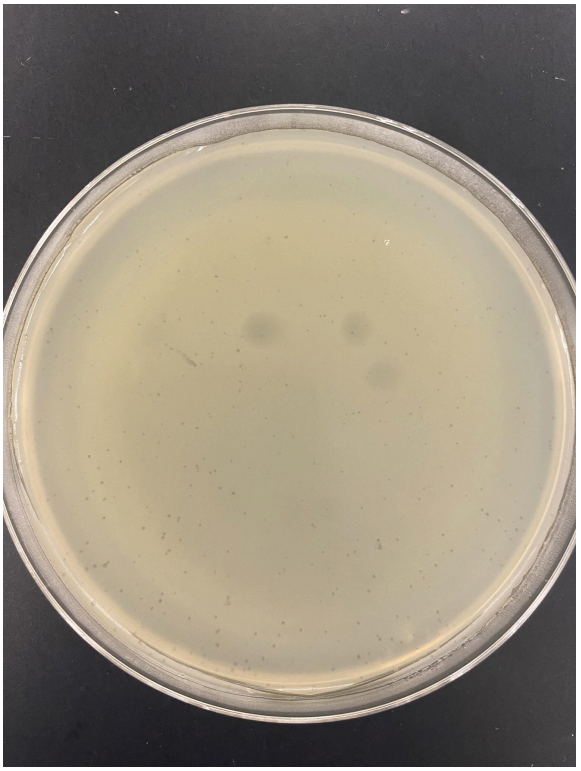
In scientific notation: 5.32x10^5 PFU/mL

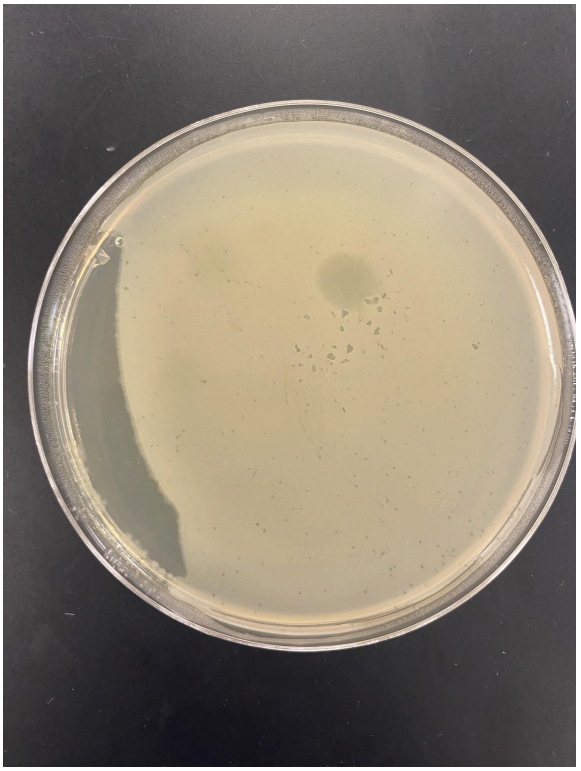
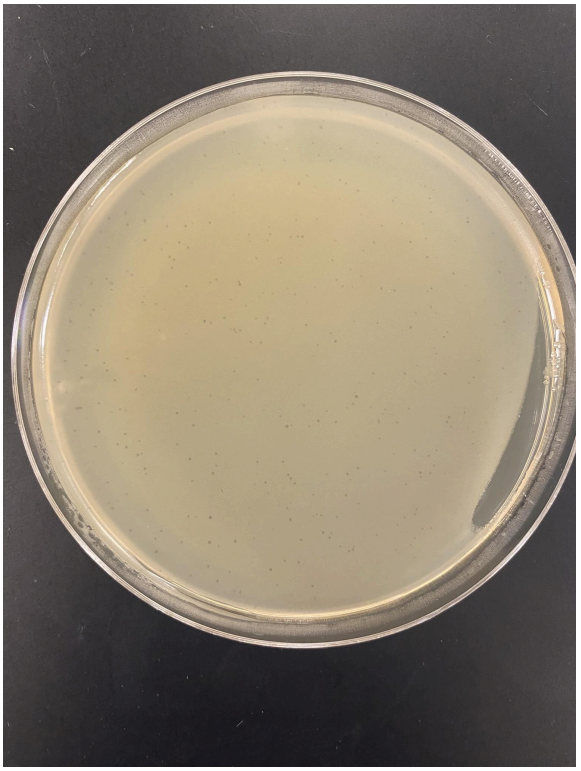
Mar 21 2025

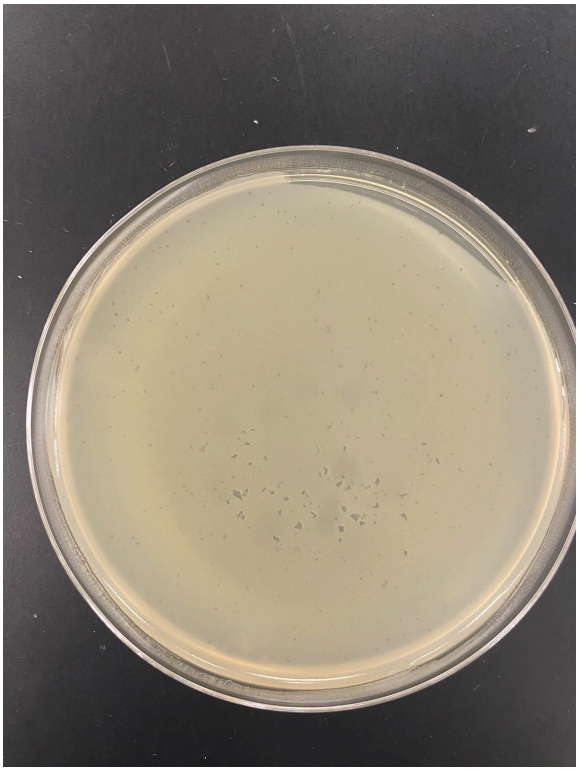
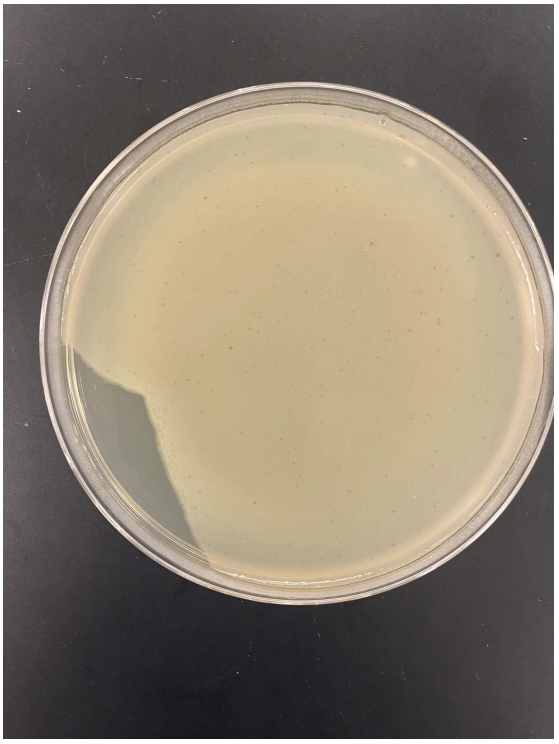
- Gathered data from plaques and formatted into charts/tables

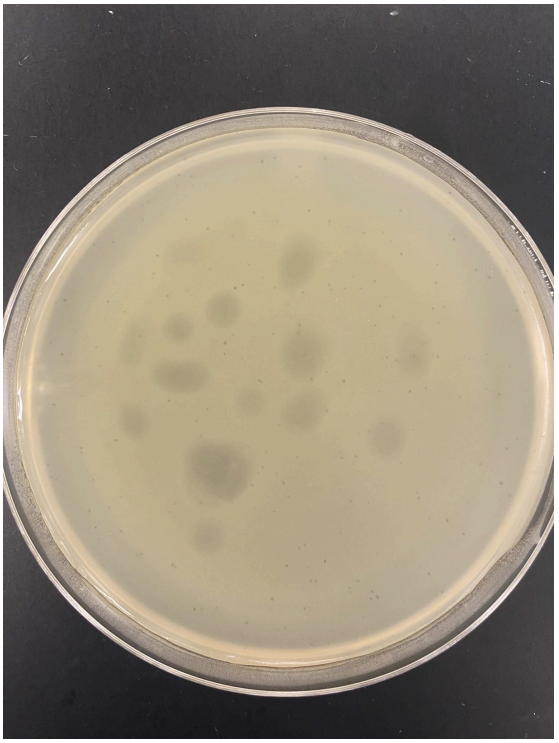
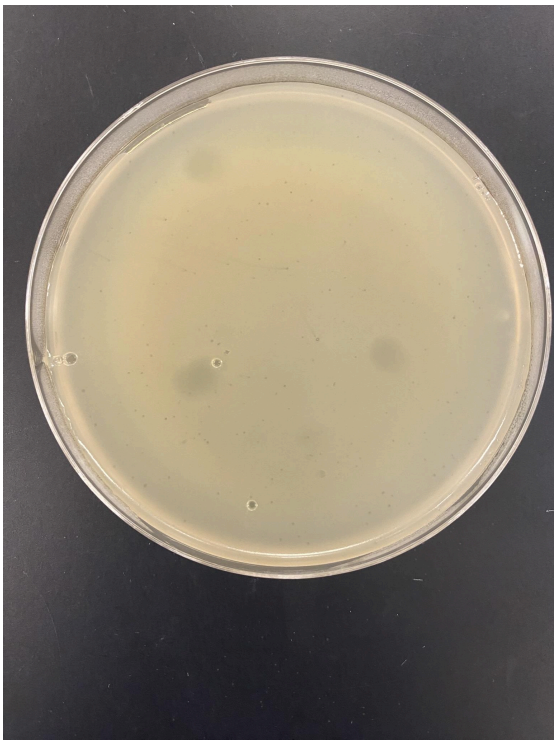
pH	Triplicate	Photo	Notes
11	1		Number of plaques: 540 Small plaques, large circles are air bubbles
11	2		Number of plaques: 224 Small plaques, large circles are air bubbles

11	3		Number of plaques: 368 Small plaques, no air bubbles or inconsistencies in plate
7	1		Number of plaques: 388 Small plaques, no air bubbles or inconsistencies in plate. More prominent compared to other 2 for pH 11

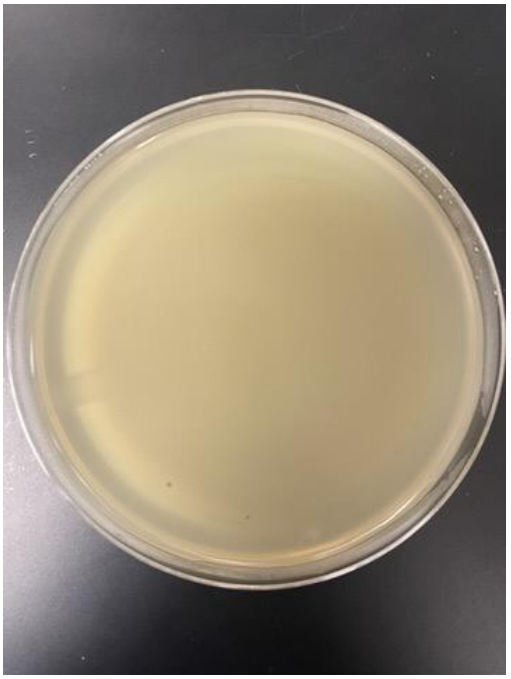
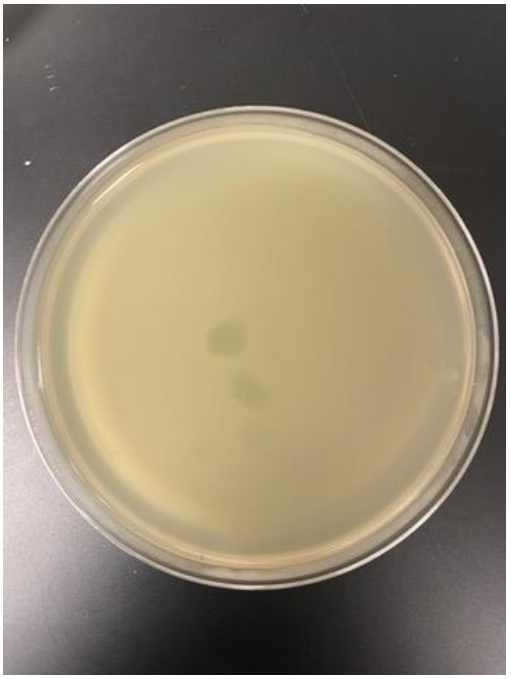
7	2		Number of plaques: 340 Small plaques, some large circles which are air bubbles
7	3		Number of plaques: 816 Small plaques, large circles as air bubbles

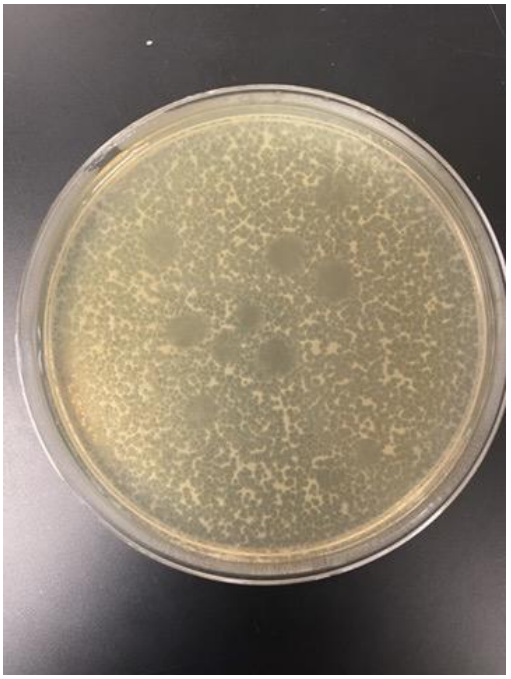
3	1		Number of plaques: 500 Small plaques, one large circle as air bubble, inconsistency in plating
3	2		Number of plaques: 396 Small plaques, inconsistency in plating

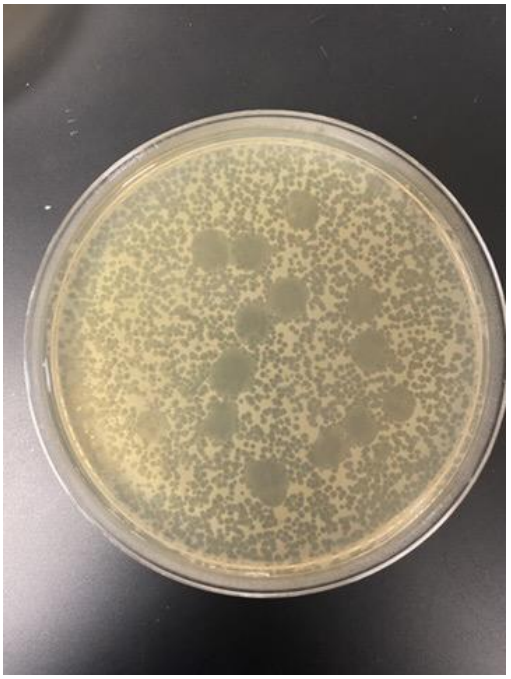
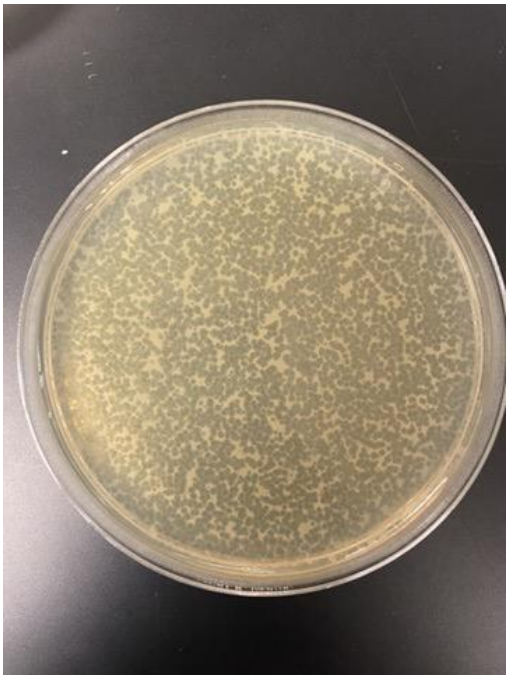
3	3		Number of plaques: 292 Small plaques, inconsistency in plating
Control	1		Number of plaques: 420 Small plaques, inconsistency in plating

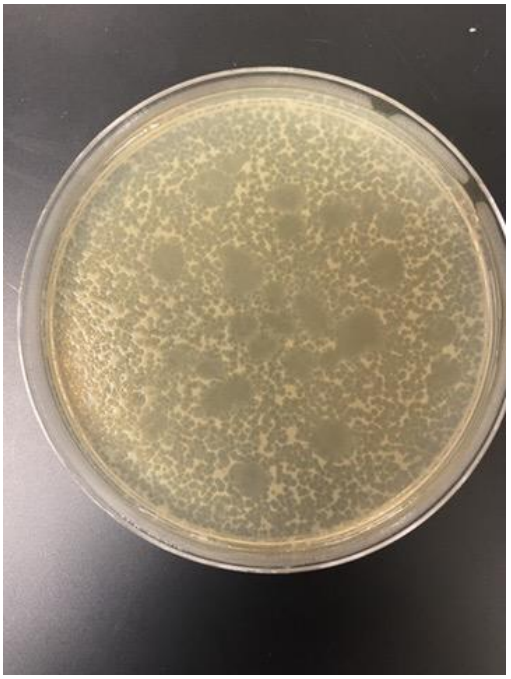
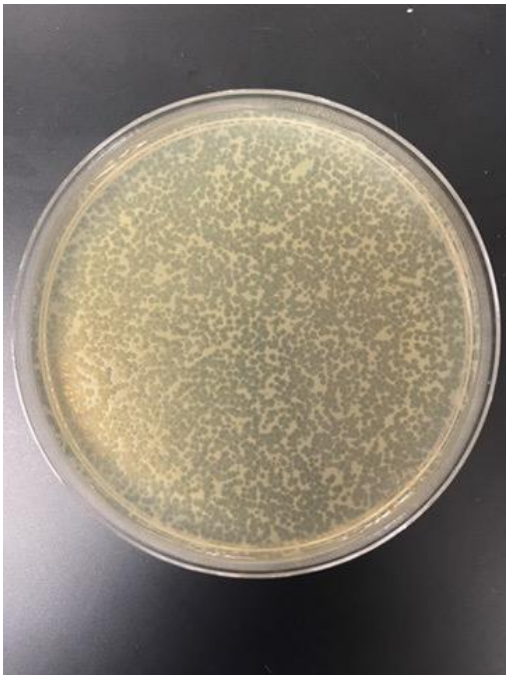
Control	2		Number of plaques: 300 Small plaques, large circles as air bubbles
Control	3		Number of plaques: 208 Small plaques, large circles as air bubbles, inconsistency in plating

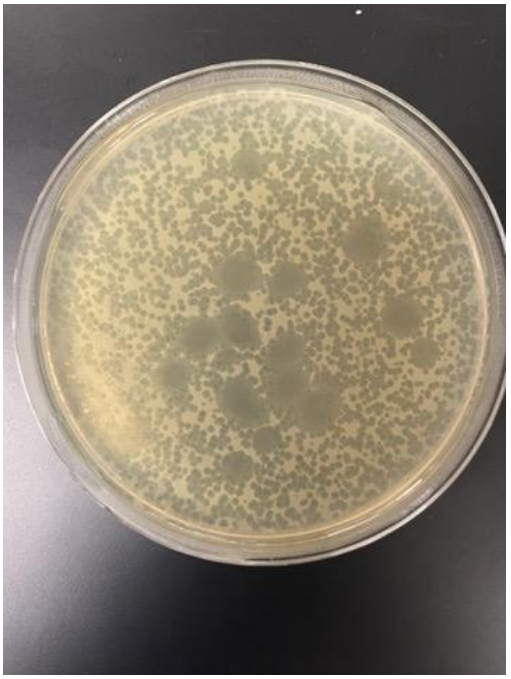
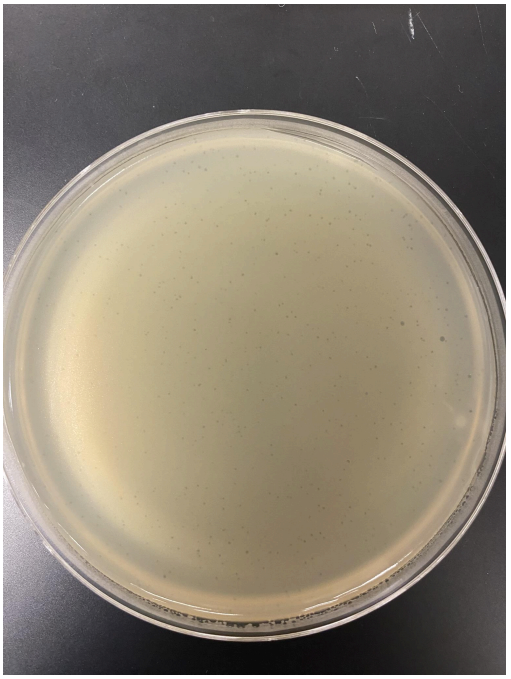
Temperature	**Triplicate**	**Photo**	**Notes**

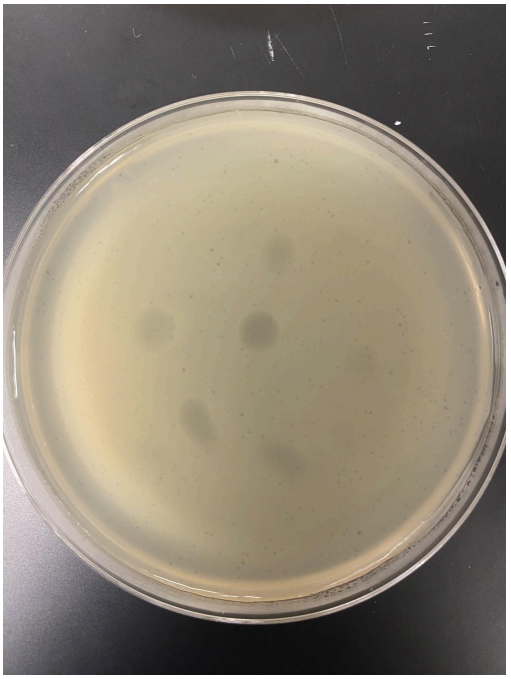
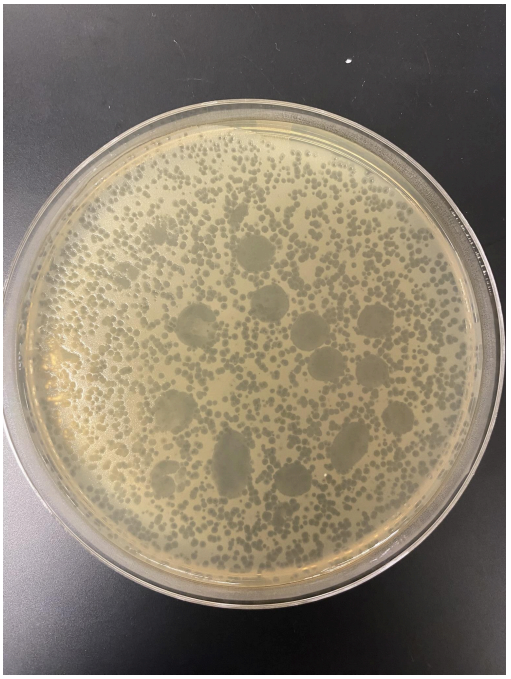
60℃	1		Number of plaques: 2 Small plaques, inconsistency in plating
60℃	2		Number of plaques: 2 Small plaques, large circles as air bubbles

60℃	3		Number of plaques: 2 Small plaques, large circles as air bubbles
37℃	1		Number of plaques: estimate 5000 based on previous plaques Small plaques with larger yield. Larger circles as air bubbles

37°C	2		Number of plaques: estimate 5000 based on previous plaques Small plaques with large yield. Large circles as air bubbles
37°C	3		Number of plaques: estimate 5000 based on previous plaques Small plaques with large yield

4℃	1		Number of plaques: estimate 5000 based on previous plaques Small plaques with large yield, large circles as air bubbles
4℃	2		Number of plaques: estimate 5000 based on previous plaques Small plaques with large yield

4℃	3		Number of plaques: estimate 5000 based on previous plaques Small plaques with big yield, large circles as air bubbles
Room Temperature	1		Number of plaques: 716 Small plaques

Room Temperature	2		Number of plaques: 636 Small plaques, large circles as air bubbles
Room Temperature	3		Number of plaques: estimate of 2000 based on previous plaques Small plaques with big yield, large circles as air bubbles. Outlier in room temperature tests

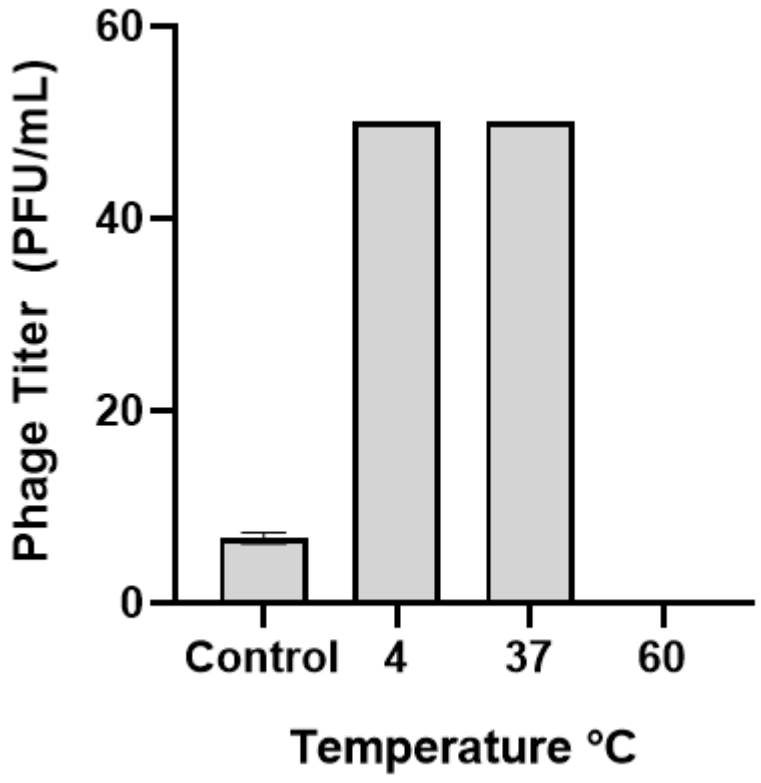
- Temperatures 4℃ and 37℃ were the most optimal temperatures for phage infection success based on our experiment
- Plaques in those temperature ranges were most abundant compared to other tested temperatures
- In the room temperature test, trial 3 from the triplicate was an outlier because it yielded an estimated 2000 plaques compared to its other two trials yielding 600-700 roughly

Our graph calculations:

New Average	New Standard Deviation
676	56.56854249
5000	0
5000	0
2	0

PFU/mL	PFU/mL (StDev)
676000	56568.54249
5000000	0
5000000	0
2000	0

10^5	
6.76	0.57
50	0
50	0
0.02	0



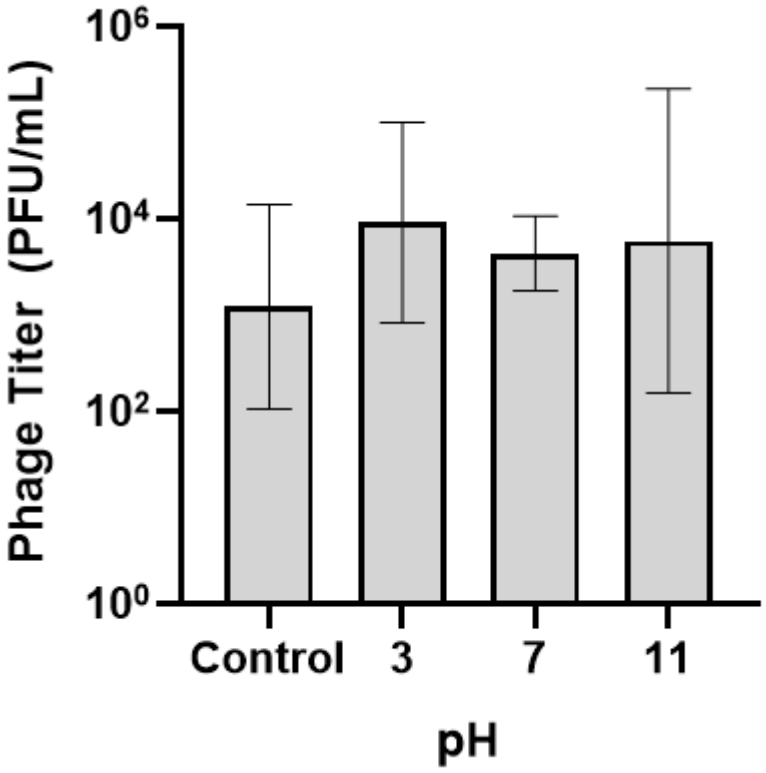
- pH level 3 was the most optimal for the phages from our experiment, but not by much

- All the pH levels tested had both high and low plaque counts in each of the trials
- pH 3 was the most consistent

New Average	New Standard Deviation
309.3333333	106.3077294
396	104
364	33.9411255
377.3333333	158.206616

PFU/mL	PFU/mL (StDev)
309333.3333	106307.7294
396000	104000
364000	33941.1255
377333.3333	158206.616

10^5	
3.09	1.06
3.96	1.04
3.64	0.39
3.77	1.58



All calculations: pH and Temperature Stability of T4 Bacteriophage

- Finished recording video and writing report, as well as updating our online platform