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Testing the Stability and Infectivity Levels of T4 Bacteriophages in Various Environments Varying in Temperature and pH

Introduction

The antibiotic resistance crisis is a problem developing in our world at a fast rate, where bacteria develop varying levels of resistance to antibiotics. It is estimated that by 2050, over 40 million deaths will occur due to the antibiotic resistance crisis. Phage therapy has been researched by experts to be one of humanity's final solutions to combating the antibiotic resistance crisis. Because phages, used in phage therapy, are biological organisms, it makes it very difficult to be FDA-approved, creating many obstacles along the way. One of those obstacles is the stability of phages, both during infection and while being stored/transported. But, before we are able to target the pain points in phage stability with tools like gene editing, we need to know what are phages' current optimal and non-optimal conditions. Our experiments aim to identify current optimal temperatures and pH levels for phages as well as the conditions in which phages are at risk for T4 bacteriophages.

Procedures

We ran 2 procedures for our T4 stability experiments. The first procedure had some flaws that made it unrealistic to gather results.

1st Procedure

We ran a total of 24 stability experiments, testing 3 temperatures and 5 pH levels. First, we grew our E. coli cultures by inoculating a single colony of E. coli into a test tube containing 5mL of LB broth and put them in an incubator set to 28℃ for 4 days. We then

had to measure and unify all the E. coli cultures to OD600 using a spectrophotometer, diluting with LB broth. The OD600 levels that were acceptable for our experiment ranged from 0.08 to 0.12, and all of them were able to fall within that range. We prepared the pH buffers using PBS (our buffer solution) and hydrochloric acid and sodium hydroxide to change the pH of the solution to the desired level. We then prepared our tests by adding 1 mL of E. coli culture and 100 µL of T4 phages stock to a solution. For the temperature tests, we left the solution as is and exposed them to either 20℃, 37℃, or 60℃. For our pH testing, we took the solutions and added 1 mL of the corresponding pH-adjusted buffer solution, varying at levels 5, 6, 7, 8, and 9. They were all exposed for 30 minutes. We then prepared the solutions to be plated by adding each solution to molten agar and then pouring that molten agar with the solution onto a plate. We then let them solidify and incubated them at 37℃ for 2 days. Then, we came in after the incubation period to gather results.

2nd Procedure

We first cultured our E. coli by inoculating a single colony of E. coli into test tubes containing 5 mL of LB broth and put them in an incubator for ~18 - 22 hrs at 37℃ with shaking at 250 revolutions per minute (rpm). The phage was prepared with incubation at 37℃ for 18 hrs with 250 rpm and then put into a centrifuge for 10 minutes at 10000 x g. Then, we prepared the pH buffer solutions using PBS (our buffer solution) and hydrochloric acid and sodium hydroxide to change the pH levels, which were going to be either pH 3, 7, or 11. We then cultured 3 plates with phages and bacteria to gather an average concentration of phages. Then, we conducted our pH and temperature tests by

preparing diluted phage solutions and exposing 100 µL of them either to the chosen pH level or to the temperatures 4℃, room temperature, 37℃, and 60℃. We then prepared the E. coli to be plated with the various treated phages, as well as a control group of phages. Then, we plated and incubated all our treated phages in triplicate for 1 day at 37℃. Then, we counted the plaques and determined the PFU/mL for each condition.

Results

When looking for phage-related results, you need to identify plaques, which are places where the phage successfully infected the bacteria. In our first trial, we were not able to detect any plaques on the petri dish surfaces. 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. Also, since there was around an equal concentration of bacteria, we can see from the varying colonies that were produced either when the phages were more/less active, where the conditions were more/less optimal for growth, and where the bacteria were able to survive the phages, although we wouldn't be able to validate our assumptions.

The results from our second procedure showed us that temperatures 4℃ and 37℃ were most optimal for phage infection, with plaque estimates being around 5000 based on other plates counted. For pH levels, pH 3 was the most optimal for phage infection. Our

results somewhat contradict what we found in papers on phage stability. For temperature results, our values align with the results of others performing phage stability tests, although it was interesting to see that at room temperature, phages did not infect at a higher level matching that of 4℃. For pH results, it was interesting to see that pH 3 performed better compared to pH 7, which in most papers was said to be most optimal for phage infection. But, pH levels 7 and 11 still were somewhat optimal for phages, but they were inconsistent with high plaque values.

Conclusion

Our results have shown us the optimal environments that T4 phages thrive at as well as the pain points in their stability in varying environments. This will allow us to further the progress in phage therapy research as well as to identify where in phage therapy enhancement we need to target our resources and efforts. Our varying results compared to other publications and papers shows that there needs to be more research and experimentation on T4 phages in order to come up with a comprehensive understanding of phage stability, from its success in infection to its stability in storage.

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Citations

Asmare, Z. (2024, August 13). *Antimicrobial resistance profile of Pseudomonas aeruginosa clinical isolates from healthcare-associated infections in Ethiopia: A systematic review and meta-analysis*. PMC. Retrieved March 6, 2025, from https://pmc.ncbi.nlm.nih.gov/articles/PMC11321567

CDC. (2019). *Multidrug-Resistant Pseudomonas Aeruginosa* [Infographic]. CDC. Retrieved March 6, 2025, from https://www.cdc.gov/antimicrobial-resistance/media/pdfs/pseudomonas-aeruginosa-508.pdf

Haenssgen, M. J. (2018, November 1). The Consequences of AMR Education and Awareness Raising: Outputs, Outcomes, and Behavioural Impacts of an Antibiotic-Related Educational Activity in Lao PDR. *Antibiotics*, *7*(4), 95. https://doi.org/10.3390/antibiotics7040095

Keen, E. C. (2016, January 1). *A century of phage research: Bacteriophages and the shaping of modern biology*. A century of phage research: Bacteriophages and the shaping of modern biology. Retrieved March 6, 2025, from https://pmc.ncbi.nlm.nih.gov/articles/PMC4418462/

Mcvay, C. S. (2007, March 26). *Phage Therapy of Pseudomonas aeruginosa Infection in a Mouse Burn Wound Model*. PMC. Retrieved March 6, 2025, from https://pmc.ncbi.nlm.nih.gov/articles/PMC1891379

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

Delbruck, M., & Luria, S. (2022, January 1). *Growth and Maintenance of Escherichia coli Laboratory Strains*. PMC. Retrieved March 6, 2025, from https://pmc.ncbi.nlm.nih.gov/articles/PMC8006063/

EPA. (2024, November 4). *What is pH? | US EPA*. Environmental Protection Agency. Retrieved March 6, 2025, from https://www.epa.gov/goldkingmine/what-ph

Hopkins, E. (2022). *Physiology, Acid Base Balance*. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK507807/#:~:text=In%20the%20absence%20of%20pathological,most%20important%20being%20blood%20oxygenation

Meyer, A. (2017, December 1). Stabilization of T4 bacteriophage at acidic and basic pH by adsorption on paper. *Colloids and Surfaces B: Biointerfaces*, *160*, 169-176. https://doi.org/10.1016/j.colsurfb.2017.09.002

Chan, B. K. (2013). Phage cocktails and the future ofphage therapy. *Review Future Microbiology*, *8*(6), 769-783. https//wwwtandfonlinecom/doi/epdf/102217/fmb1347?needAccess=true

Fister, S. (2016, July 27). Influence of Environmental Factors on Phage–Bacteria Interaction and on the Efficacy and Infectivity of Phage P100. *Frontiers in Microbiology*, *7*. https://doi.org/10.3389/fmicb.2016.01152

García-Cruz, J. C. (2023, April 21). Myriad applications of bacteriophages beyond phage therapy. *PMC*. 10.7717/peerj.15272

Kasman, L. M. (2022). *Bacteriophages*. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK493185/

Laanto, E. (2020, May 29). Adapting a Phage to Combat Phage Resistance. *Antibiotics*, *9*(6). https://doi.org/10.3390/antibiotics9060291