Lab Report

# Proof of principle: utilizing bacteriophages for acne treatment*

**Alisa Koltsova** ▫ Oak Ridge High School, El Dorado Hills, CA

**Adrian Luck and Alexander Luck** ▫ BioBuilder Club, Andover High School, Andover, MA

Reviewed on 4 May 2024; Accepted on 10 June 2024; Published on 26 October 2024

*Bacteriophages are viruses that infect and kill bacterial hosts. Because of their specificity and virulence, bacteriophages are desirable candidates for treatment of bacterial infections. Given the growing problem of antibiotic resistance, phages can be used as an effective alternative against both the antibiotic sensitive and the resistant bacterial strains. In the following study, we explore the use of bacteriophages for treatment of acne. Based on the results, we were able to isolate Cutibacterium acnes-specific phages, increase virulence via ultraviolet (UV)-induced random mutagenesis, and demonstrate that phages are a potent alternative and may prove to be a successful treatment for bacterial infections.*

Keywords: Bacteriophages, acne, antibiotic resistance, bacteriophage therapy


Bacteriophages (also known as phages) are viruses that infect bacteria. Phages are the most abundant organisms and are a ubiquitous feature of bacterial existence (Abedon, 2018). They are often found naturally near their host or integrated within the host's genome. Once a phage infects a susceptible host, it may pursue one of two cycles of replication: lysogenic or lytic (Figure 1) (Bond et al., 2021; Luck et al., 2022; Slipe et al., 2023). During the lysogenic cycle, the phage DNA is incorporated into the host genome, where it lays dormant and is passed on to next generations. In contrast, in the lytic cycle, the phage hijacks the host's cellular machinery to replicate and propagate, killing its host and infecting neighboring host cells. Environmental stressors that affect the host, such as malnutrition, dramatic changes in pH or temperature, exposure to toxic chemicals or ultraviolet light (UV) irradiation, may induce the lytic cycle and force the prophage to excise from the cell and seek viable hosts to ensure survival (Bond et al., 2021; Clokie et al., 2011; Luck et al., 2022; Pires et al., 2021; Slipe et al., 2023).

In the era when antibiotic resistance is common, bacteriophages offer a solution and alternative method of treating bacterial infections. In the U.S., more than 2.8 million antimicrobial-resistant infections occur each year (CDC, 2019). Overuse and incorrectly prescribed antibiotics are the major reasons for proliferation of bacterial resistance (CDC, 2013; Ventola, 2015). There are also many instances when antibiotics are prescribed without proper testing for presumptive infections (Ventola, 2015). In some countries antibiotics are unregulated and can be

* *The authors were mentored by Barbara Tevelev from Andover High School. Please direct correspondence to: btevelev@gmail.com.*

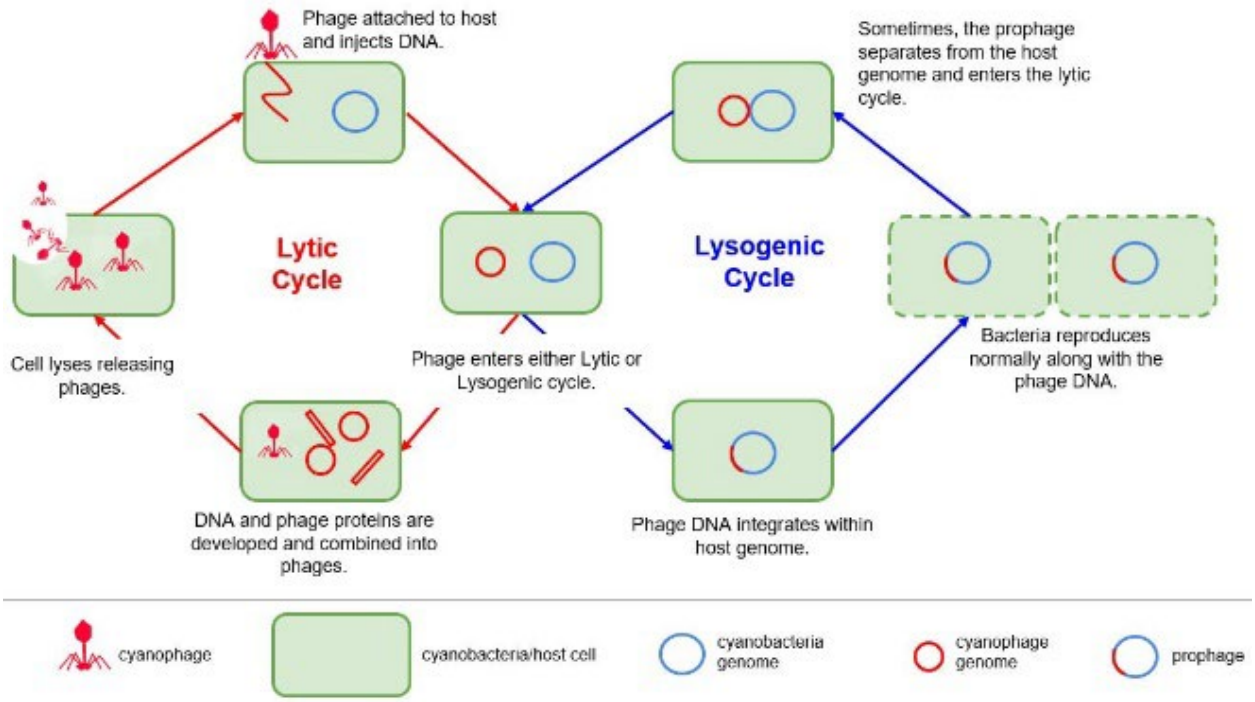


*Figure 1. Bacteriophage Lytic and Lysogenic Cycle (Duplicated from our previous design brief in BioTreks: https://biotreks.org/wp-content/uploads/2022/10/e202202.pdf. Image credit: Adrian Luck.*

purchased over the counter or online (Michael, et al., 2014). Furthermore, inappropriate prescribing which includes errors in selected treatment, choice of antibiotics, as well as duration and dosage contributes to resistance (CDC, 2013). Antibiotic resistance has been declared by many health organizations as a "crisis" or "nightmare scenario" that can lead to "catastrophic consequences" (Viswanathan, 2014). Multidrug resistance (MDR) bacteria have already proved disastrous and fatal (Golkar, 2014; Ventola, 2015). *Staphylococcus aureus,* which is resistant to methicillin, aminoglycosides, macrolides, tetracycline, chloramphenicol, lincosamides, as well as disinfectants (MRSA) can be life-threatening and a major source of hospital-acquired infections (Golkar, 2014; de Lencastre, 2007). Thankfully, pharmaceutical companies were able to develop agents to treat MRSA such as linezolid and quinupristin/dalfopristin (Shariati, 2020). Unfortunately, the arms race between pathogens and hosts continues, and there are some gram-negative bacteria that have already evolved resistance to most of the newly developed agents (Breijyeh et al., 2020; Livermore, 2004; Miller, 2016). The instances of such resistance occurred recently, so the development of new antibacterial agents is lagging behind (Exner et al., 2017; Livermore, 2004; Ventola, 2015).

Focusing research efforts on bacteriophages may help to solve the crisis of bacterial antibiotic resistance. Phages infect and kill bacteria without any negative effect on human or animal cells, so they can be used alone to treat infections or in combination with antibiotics to enhance treatment (Domingo-Calap and Delgado-Martínez, 2018). Bacteriophage therapy was often used in the Soviet Union in patients suffering from all types of bacterial infections and the results were largely positive (Summers, 2001). Unfortunately, the Soviet reports were not translated nor well-regarded in the West and ignored due to the popularity of antibiotics after World War II (Chanishvili, 2016). Given the rise of multi-drug-resistant bacteria and a decline in the development of novel antibacterial agents, bacteriophages are being reconsidered (Perros, 2015; World Health Organization, 2018).

Besides being bactericidal, phages

present many additional advantages. Due to their specificity, phages do not impact health-protecting normal flora of bacteria (Skurnik et al., 2007; Gupta et al., 2001 and 2019). Their relatively narrow host range limits the number of bacterial types with which selection for specific phage-resistance mechanisms can occur (Hyman and Abedon, 2010). Also, acquired phage-resistance by the host has been shown to negatively impact bacterial fitness due to loss of pathogenicity-related phage receptors, increasing bacterial mortality (Capparelli, 2010). Phages multiply their numbers specifically where hosts are located, so they establish appropriate "auto" dose and respective clearance levels as their proliferation is self-limiting (Capparelli, 2010; Loc-Carrillo and Abedon, 2011). Phages are mostly composed of nucleic acids and proteins that are inherently non-toxic, but the bacteria they lyse may contain harmful toxins so anaphylactic responses to bacterial components must be monitored (Skurnik et al., 2007; Hyman and Abedon, 2010; Loc-Carrillo and Abedon, 2011). Lastly, unlike antibiotics, phages are easier to isolate, grow and genetically manipulate, hence facilitating rapid discovery of antibacterial alternatives and providing treatments for a variety of diseases, such the one explored in this paper - acne (Clokie et al., 2011; Loc-Carrillo and Abedon, 2011).

Acne is a common skin condition where pores in the skin become clogged. Sebum, which keeps skin from drying out, along with dead skin and common skin bacteria such as *C. acnes* clogs the pores and leads to inflammation and formation of nodules or pustules, otherwise known as pimples (Mayslich, at al., 2021; Cavallo et al., 2022). *C. acnes* and its components trigger the immune response which activates white blood cells in the infected area and results in an inflammatory reaction, characterized by swelling and redness. See Figure 2 for how acne develops. By eliminating *C. acnes*, the inflammatory response may be avoided or largely decreased along with discomfort and unattractive appearance. Common treatments for acne include sialic acid, benzoyl peroxide, and antibiotics such as tetracycline or macrolide. The obvious disadvantage of these treatments is exposure to chemicals and potential antibiotic resistance when used over a prolonged period of time. Using bacteriophages would eliminate the use of

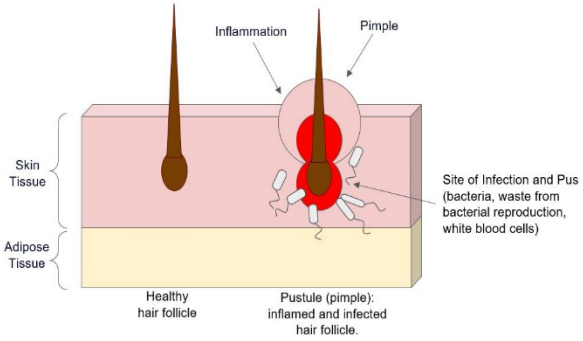


*Figure 2. Pathogenesis of Acne. The onset of acne is a multifactorial process that involves stimulation of sebaceous glands, plugging of the follicle, excess sebum production, presence of C. acnes and inflammation. Credit: Adrian Luck.*

toxic chemical products that could lead to skin sensitivities and eczema as well as would prevent overuse of antibiotics, alleviating the crisis of antibiotic-resistance.
In the following study, we explore the use of bacteriophages for treatment of acne as part of proof of principle that phages are viable bactericidal agents. First, a bacterial host, *C. acnes*, was isolated from facial areas. Given the high probability that many bacteria contain a dormant phage, UV was used to induce the lytic cycle, and phages were collected from the observed plaques, which are clearings in the bacteria lawns. Consecutively, the collected phages were subjected to UV radiation to create random mutations in the phage genome. The phage mutants were tested against the isolated host to determine level of virulence as represented by plaque size and frequency. Results indicated that random mutations increased virulence and thus the phage capability to kill *C. acnes,* suggesting that phages may be a potential treatment for acne. Overall, we were able to show that bacteriophages can be utilized as a possible alternative to antibiotics, increasing the medical arsenal for combating bacterial infections.

# Materials and methods

Materials and equipment used in the experiments are listed Table 1. Design for this research project is depicted in Figure 3.

***Table 1. List of Materials and Equipment Used During Experiments.***

| Reagents | | | |
|---|---|---|---|
| **Item** | **Vendor/ID** | **Description** | **Use** |
| Blood Agar Plates | Diamante Scientific, Youngstown, OH | TSA with 5% Sheep Blood (10-Pack). Pre-Poured, 15x100mm Plates | Bacterial and phage culture. |
| Saline solution | UFC Bio, Amherst, NY | 0.9% Normal Saline Solution - 0.22um Filtered and Sterile | Collecting bacteria and phages. |
| L-shaped Spreaders | Argos Technologies, Vernon Hills, IL | C4050 Polypropylene Sterile Disposable Blue | Spreading bacterial culture over the plates. |
| 70% Ethanol | Volu-Sol, Salt Lake City, UT | Denatured Ethanol SDA 70% | Disinfecting surfaces and instruments before and after experiments. |
| Gram Stain Kit | Home Science Tools, Billings, MT | Crystal violet stain, Gram iodine stain, Ethyl alcohol solvent Safranin counterstain, Plain microscope slides, Medicine dropper, Coverslips | Host identification |
| **Equipment** | | | |
| **Item** | **Use** | | |
| UV lamp with UV protector | Phage induction and mutagenesis | | |
| Vortex | General use during experiments; re-suspend cells and phages; mix reagents | | |
| Centrifuge | General use during experiments; spin down cell and phages | | |
| Pipetteman (P20, P200, and P1000) | Accurately measuring and transferring small amounts of solutions | | |
| Microscope | Identify host | | |
| **Safety Equipment** | | | |
| **Item** | **Use** | | |
| Safety glasses | Protective laboratory gear | | |
| Gloves | Protective laboratory gear | | |
| Cleaning wipes (bleach) | Disinfect surfaces before and after use | | |

## *Experiment 1: Host Isolation*

A possible isolate of *C. acnes* was collected from the researcher's skin. A cotton swab was used to rub along the outside of the nose several times. The collected samples were transferred onto blood agar plates by gently rolling the cotton tip across the surface of the agar. Four samples were taken. All the plates were wrapped with parafilm to simulate anaerobic conditions and left for ten days at room temperature (RT). Only one plate displayed growth and was used for Experiment 2.

The plate with growth was analyzed to determine if the isolates may be *C. acnes*. Gram stain was performed as an identification method. A colony was picked with a loop and transferred onto a slide and mixed with water. Heat was used to fix the slide and after cooling, stained with crystal violet for 1 minute and rinsed with water. Next, iodine solution was applied for 1 minute, rinsed with water and finally flooded the slide with a decolorizer for 3 seconds and immediately rinsed with water. The slide was examined under a microscope.

## *Experiment 2: Growing Bacterial Lawns*

The plate with possible isolates of *C. acnes* colonies was washed with saline solution to collect and combine all of the colonies. A heterogeneous solution of different colonies was desirable as a substrate for bacterial lawns. The collected cells were transferred into an Eppendorf tube and gently mixed by flicking so as not to cause premature cell lysis. Approximately 250 microliters of the resuspended solution was then transferred on the new blood agar plates and evenly spread with a L-shaped spreader. Next, the plates were wrapped in parafilm to simulate anaerobic conditions and placed in an incubator at 37 degrees celsius for 7 days. Once the plates were overgrown with bacterial lawns, Experiment 3 was carried out.

## *Experiment 3: Phage Induction*

It is expected that most bacteria genomes contain a prophage. Prophages are dormant phages that are integrated in the host's genome and replicate when the host divides. Under environmental stressors, the prophage will enter the lytic cycle and excise from the host. An environmental stressor was simulated by exposing the bacterial lawns to UV radiation. Multiple durations of UV exposures were tested including 20, 30, 60,

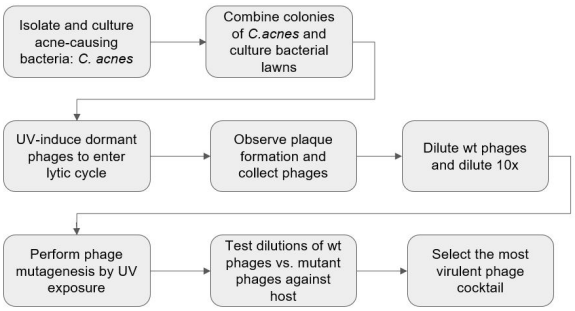


*Figure 3. Research Strategy and Workflow. Image credit: Alexander and Adrian Luck.*

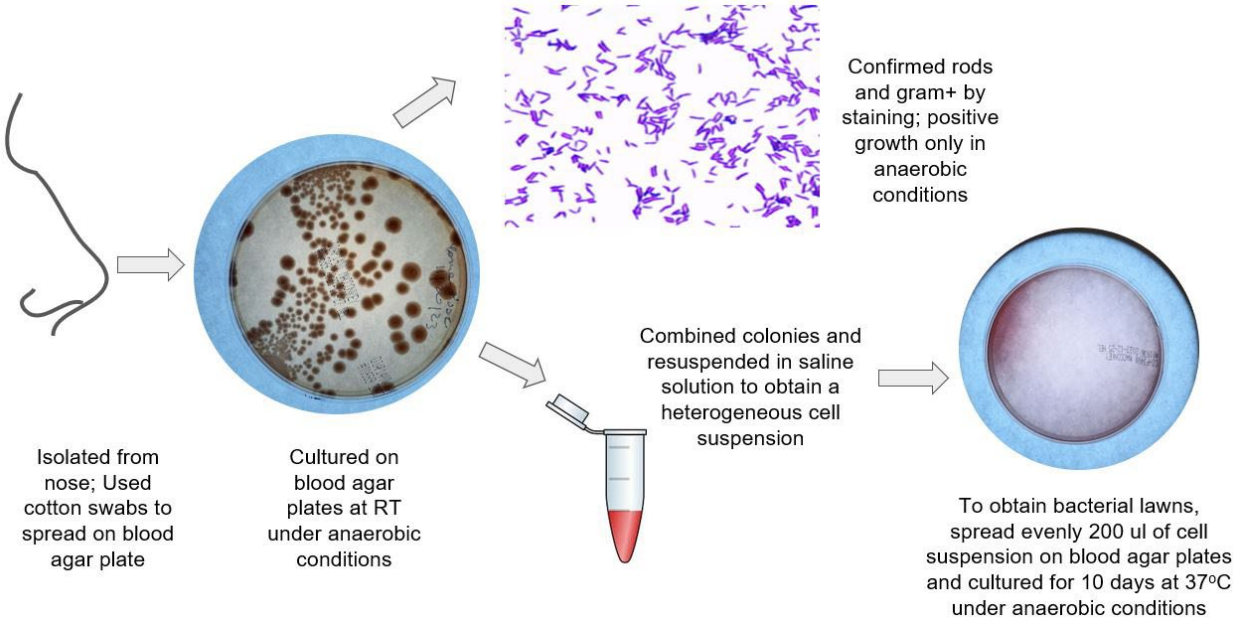


*Figure 4. Host Isolation and Identification. Sample of bacteria was collected from skin and grown on blood agar plates at room temperature (RT) and under anaerobic conditions. The cultured colonies were analyzed by staining techniques and further grown. Image credit: Alexander and Adrian Luck.*

and 90 seconds. Plaques were observed over the course of 2 days. To collect phages, plaques were washed with saline solution. The saline solution with phages and cell debris was aspirated, centrifuged to eliminate host cells and debris, and the supernatant was transferred to a new Eppendorf tube. A total of 4 phage supernatants were collected: P1wt, P2wt, P3wt and P4wt. The "wt" refers to the initial wildtype phage.

### *Experiment 4: Phage Mutagenesis*

Approximately 200 microliters from each of the four wt phage supernatants were subjected to UV radiation at different time intervals: 30, 60, and 120 seconds. After irradiation, 20 microliters of the mutated phage solution was placed in one of the four quadrants on the bacterial lawn plate to determine infection rate. Each of the mutated phage solutions was spread evenly on the corresponding section of the plate. As a positive control, the wt was included during re-infection. The plates were then wrapped in parafilm and left at RT. Plaques were observed on day 2 and results recorded.

# Results

### *Experiment 1: Host Isolation and Identification*

A *C. acnes* potential isolate was successfully cultured to sufficient numbers of colonies under anaerobic conditions. According to the gram stain test, gram positive bacteria remain purple, while gram negative bacteria turn pink or red. The isolated bacteria turned purple and appeared rod-shaped under the microscope (Kurihara et al., 2023). These observations, together with growing only in anaerobic environment, are consistent with *C. acnes* phenotype (Kurihara et al., 2023). The isolated colonies were used as a substrate for Experiment 2. See Figure 4. Lack of additional equipment and funding to initiate genotypic characterization limited identification techniques, and so the identity of *C. acnes* was not fully confirmed.

### *Experiment 2: Growing Bacterial Lawns*

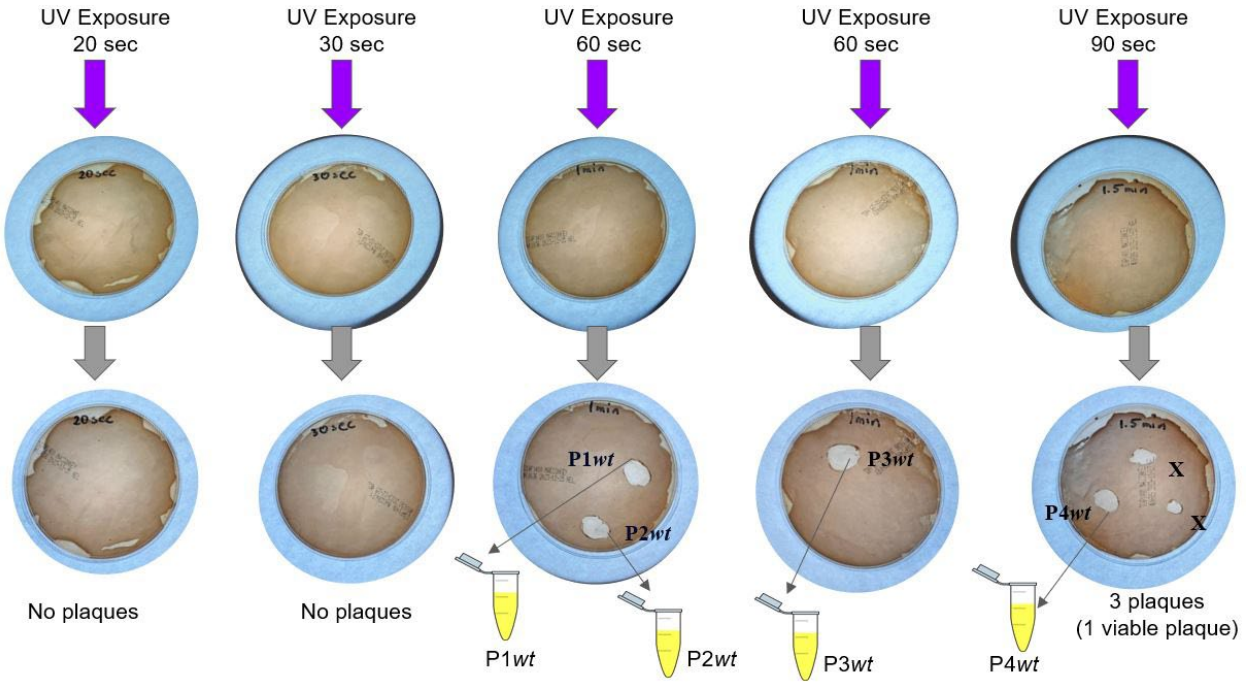


*Figure 5. Phage Induction via UV Exposure. Bacterial lawns were exposed to UV (row 1) and plaques were identified and collected (row 2). Image credit: Alexander and Adrian Luck.*

Bacterial colonies from Experiment 2 (Figure 4: plate with multiple distinct colonies) were pooled and cultured. After a 7-day incubation period at 37 degrees Celsius, bacterial lawns were observed across all plates. The growth was robust and evenly spread across the plates (See Figure 5: top row of plates).

### *Experiment 3: Phage Induction*

Once the plates with bacterial lawn were exposed to UV at different time durations, plaques were observed after two days (see Figure 5: bottom row of plates). Phages isolated from these plaques were referred to as wt. Plates exposed to 60 seconds of UV contained two plaques (P1wt and P2wt). Another plate exposed to UV for 60 seconds contained a single large plaque (P3wt). The third plate, exposed for 90 seconds to UV yielded 3 plaques; two of which were smaller in size and irregularly shaped and so were eliminated from further study, while the third plaque (P4wt) was selected for further experimentation. The phages were isolated from the plaques using a saline solution wash.

### *Experiment 4: Phage Mutagenesis*

In the phage mutagenesis experiment, UV irradiation was used to create random mutations in the collected phage solutions from experiment 3. Four wt phage solutions (P1wt, P2wt, P3wt, and P4wt) were exposed to three different time intervals of UV: 30 sec, 60 sec and 120 sec. New bacterial lawn plates were divided into 4 quadrants. The wt control (not exposed to another round of UV) and three flavors of irradiated phages (mutants) were plated onto each respective quadrant.

For P1, no plaques were observed in the wt control nor in any of the quadrants with mutated phage.

For P2, a single plaque was observed in the wt control and two larger plaques were observed in the 30 sec quadrant. No plaques were observed at either 60 or 120 seconds.

For P3, two plaques were observed in the wt control, three plaques were observed in the 30 sec quadrant and a single plaque was observed in the 60 sec quadrant. No plaques were observed at 120 sec.

For P4, three plaques were observed for wt control, three plaques in the 30 sec

quadrant, and six plaques in the 60 sec quadrant. No plaques were observed in the 120 sec quadrant.

Based on the results, the most virulent phage is the mutant generated from P4 exposed to 60 sec UV light (Figure 6). The 60 sec quadrant had not only the most amount of plaques but relatively largest plaques. The second virulent phage was a tie between mutant P3 and P4 at 30 sec UV exposure. Across all plates, the 120 sec quadrants were intact, indicating that perhaps that duration of UV destroyed or damaged the phage and prevented reinfection.

# Discussions

Antibiotics are frequently used to treat acne. Initially, these treatments are successful, however, they inevitably result in increased bacterial resistance. Utilizing bacteriophages to treat bacteria-causing diseases, like acne, is promising and effective. Furthermore, bacteriophages are chemical-free alternatives that are considered hypo-allergenic and nontoxic. And so, using bacteriophage-related treatments on skin may prevent various secondary epidermal irritations.

The following study demonstrated that it is possible to isolate phages from acne-causing bacteria and increase their virulence by random mutagenesis. Although the following research was able to establish proof of principle for use of bacteriophages as a potential bactericidal agent, additional experiments are needed to optimize the process of mutagenesis.

As mentioned in previous study on cyanophages (Luck et al., 2022), to improve upon the current idea, it would be preferable to control location of mutations rather than relying on a random process of mutagenesis. For example, identifying genes associated with lysogenic cycle and knocking them out or identifying and enhancing genes associated with the lytic cycle will ensure increased phage virulence. Utilizing techniques such as CRISPR would yield more control and precision during genetic manipulation.

A challenge that may hinder effectiveness of bacteriophage therapy is presence of a latent phage within the host's genome. Hosts

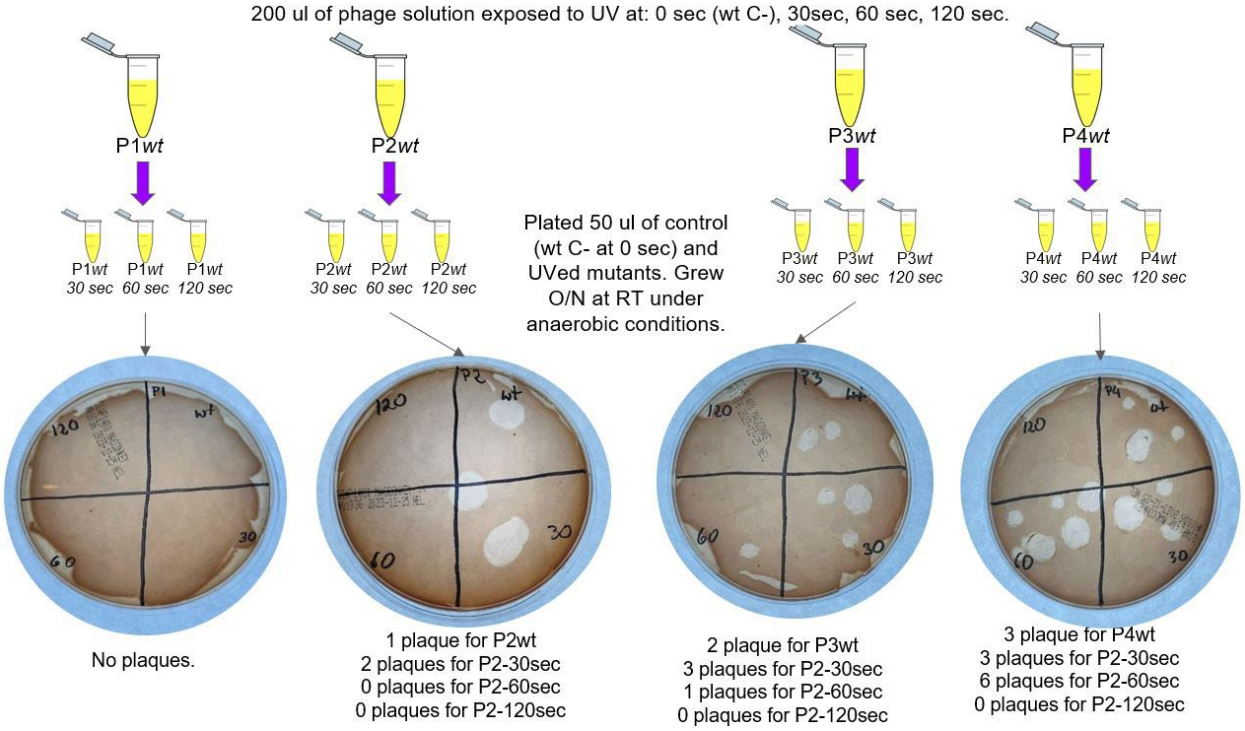


*Figure 6. Phage Mutagenesis via UV Exposure. Wild type phage solutions isolated from experiment 3 were exposed to UV to generate random mutations. C. acnes isolates were reinfected by the mutated phages and plaques were observed. Image credit: Adrian and Alexander Luck.*

that already contain a phage integrated within their genome may be immune to subsequent superinfection (Bondy-Denomy et al., 2016). Some phages have an evolved mechanism that alters the bacterial genotype to make it resistant to phage superinfection. There are no systematic studies to elucidate the mechanism of superinfection, and the process is poorly understood (Bondy-Denomy et al., 2016). At this point, it is unclear how to solve this host immunity. One possibility is to make sure to only use lytic phages in phage therapy to prevent lysogenic incorporation and associated complications.

Nevertheless, more and more research suggests successful application of phage therapy. In the field of skin infections, several studies have already indicated the effectiveness of bacteriophage therapy (Qin et al.,2020) have shown that 95% of patients that suffered from chronic wounds and ulcers experienced remission or significant improvement from phage therapy. In another study, patients infected with *Pseudomonas aeruginosa*, which can cause serious and potentially dangerous infections in people who have a weakened immune system, were successfully cured using phages (Qing, 2007; Liang et al., 2023). Also in the same study, phage treatment was shown to be effective in patients infected with drug-resistant *P. aeruginosa* after surgery (Qing, 2007). Phage therapy is promising but such treatments should be introduced with caution until they are fully understood.

# Next Steps

The next experiments should focus on more controlled methods of mutagenesis. Identifying and knocking out the integrase gene or creating mutations that deactivate the integrase gene will increase virulence. Testing various mutants individually and in combination may be another way to increase virulence and improve infection rates across different strains of *C. acnes* from different individuals.

Another challenge that has been identified is the risk of the human immune system recognizing and reacting to the therapeutic phages (Jariah and Hakim, 2019; Mangalea and Duerkop, 2020). The interaction between human immune system, bacteria and phages must be studied in more detail in order to understand the applicability, effectiveness and safety of phage therapy. Ideally, phage treatments should produce large burst sizes, short latent periods, broad range across different host strains, tendency to prevent resistance, and no adverse health effects (Jariah and Hakim, 2019; Mangalea and Duerkop, 2020).

Ultimately, a topical treatment for *C. acnes* with bacteriophages that is effective, non-toxic, and easy to use would be ideal. Given the already established effectiveness of phages against bacteria and most importantly against antibiotic-resistant bacteria, the next research steps should focus on developing appropriate delivery systems to the infected area. A topical cream seems suitable for acne. However, it is unclear what components should constitute such a cream in order to retain phage integrity, stability, and mobility. Further feasibility studies are required.

# Author contributions

A.J.L and A.E.L came up with the idea and experimental strategy. All authors contributed equally to literature search and review. All authors contributed equally to writing and addressing comments during the review process.

# Acknowledgements

We would like to acknowledge and thank our mentor, Barbara Televev as well as our teacher, Lindsey L'Ecuyer. We appreciate their guidance, support and help. We also want to thank the AHS BioBuilderClub for providing us with the opportunity to pursue our interests in synthetic biology and bioengineering.

# References

Abedon, S.T. (2008). Phages, Ecology, Evolution. In: Abedon ST, editor. *Bacteriophage Ecology: Population Growth, Evolution, and Impact of*

*Bacterial Viruses.* Cambridge University Press; pp. 1–28.

Bond, M.C., Vidakovic, L., Singh, P.K., Drescher, K., & Nadell, C.D. (2021). Matrix-trapped viruses can prevent invasion of bacterial biofilms by colonizing cells. Shou W, Storz G, Shou W, editors. *Elife.* 10:e65355. doi: 10.7554/eLife.65355

Bondy-Denomy, J., Qian, J., Westra, E. R., Buckling, A., Guttman, D. S., Davidson, A. R., & Maxwell, K. L. (2016). Prophages mediate defense against phage infection through diverse mechanisms. *The ISME journal*, *10*(12), 2854–2866. https://doi.org/10.1038/ismej.2016.79

Breijyeh, Z., Jubeh, B., & Karaman, R. (2020). Resistance of Gram-Negative Bacteria to Current Antibacterial Agents and Approaches to Resolve It. Molecules (Basel, Switzerland), 25(6), 1340. https://doi.org/10.3390/molecules25061340

Capparelli, R., Nocerino, N., Iannaccone, M., Ercolini, D., Parlato, M., Chiara, M., et al. (2010). Bacteriophage therapy of *Salmonella enterica*: a fresh appraisal of bacteriophage therapy. *J Infect Dis.* 201:52–61. doi: 10.1086/648478.

Cavallo, I., Sivori, F., Truglio, M., De Maio, F., Lucantoni, F., Cardinali, G., Pontone, M., Bernardi, T., Sanguinetti, M., Capitanio, B., Cristaudo, A., Ascenzioni, F., Morrone, A., Pimpinelli, F., & Di Domenico, E. G. (2022). Skin dysbiosis and Cutibacterium acnes biofilm in inflammatory acne lesions of adolescents. *Scientific reports*, *12*(1), 21104. https://doi.org/10.1038/s41598-022-25436-3

Centers for Disease Control and Prevention (CDC), Office of Infectious Disease Antibiotic resistance threats in the United States. (2013). Available at: http://www.cdc.gov/drugresistance/threat-report-2013.

Centers for Disease Control and Prevention (CDC). (2019.). *Antibiotic Resistance Threats in the United States.* https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf

Chanishvili, N. (2001). Phage therapy–history from Twort and d'Herelle through Soviet experience to current approaches. *Adv. Virus Res.* 83 3–40. 10.1016/B978-0-12-394438-2.00001-3

Clokie, M. R., Millard, A. D., Letarov, A. V., & Heaphy, S. (2011). Phages in nature. *Bacteriophage*, *1*(1), 31–45. https://doi.org/10.4161/bact.1.1.14942

de Lencastre, H., Oliveira, D., & Tomasz, A. (2007). Antibiotic resistant *Staphylococcus aureus*: a paradigm of adaptive power. *Curr. Opin. Microbiol.* 10:428–35.

Domingo-Calap, P., & Delgado-Martínez, J. (2018). Bacteriophages: protagonists of a post-antibiotic era. *Antibiotics* 7:E66. 10.3390/antibiotics7030066

Exner, M., Bhattacharya, S., Christiansen, B., Gebel, J., Goroncy-Bermes, P., Hartemann, P., Heeg, P., Ilschner, C., Kramer, A., Larson, E., et al. (2017). Antibiotic resistance: What is so special about multidrug-resistant Gram-negative bacteria? GMS Hyg. Infect. Control. 12 doi: 10.3205/dgkh000290.

Golkar, Z., Bagazra, O., & Pace, D.G. (2014). Bacteriophage therapy: a potential solution for the antibiotic resistance crisis. *J Infect Dev Ctries.* 8(2):129–136. 13.

Gupta, R., & Prasad, Y. (2001). Efficacy of polyvalent bacteriophage p-27/HP to control multidrug resistant Staphylococcus aureus associated with human infections. *Curr Microbiol.* 62:255–260. doi: 10.1007/s00284-010-9699-x.

Gupta, P., Singh, H. S., Shukla, V. K., Nath, G., & Bhartiya, S. K. (2019). Bacteriophage Therapy of Chronic Nonhealing Wound: Clinical Study. The international journal of lower extremity wounds, 18(2), 171–175. https://doi.org/10.1177/1534734619835115

Hyman, P., & Abedon, S.T. (2010). Bacteriophage host range and bacterial resistance. *Adv Appl Microbiol.* 270:217–248. doi: 10.1016/S0065-2164(10)70007-1.

Jariah, R. O. A., & Hakim, M. S. (2019).

Interaction of phages, bacteria, and the human immune system: Evolutionary changes in phage therapy. Reviews in medical virology, 29(5), e2055. https://doi.org/10.1002/rmv.2055

Kurihara, M. N. L., Santos, I. N. M., Eisen, A. K. A., Caleiro, G. S., Araújo, J., Sales, R. O., Pignatari, A. C., & Salles, M. J. (2023). Phenotypic and Genotypic Characterization of Cutibacterium acnes Isolated from Shoulder Surgery Reveals Insights into Genetic Diversity. Microorganisms, 11(10), 2594. https://doi.org/10.3390/microorganisms11102594

Liang, S., Qi, Y., Yu, H., Sun, W., Raza, S.H.A., Alkhorayef, N., Alkhalil, S.S., Salama, E.E.A., & Zhang, L. (2023). Bacteriophage Therapy as an Application for Bacterial Infection in China. *Antibiotics*. 12(2):417. https://doi.org/10.3390/antibiotics12020417

Livermore, D.M. (2004). The need for new antibiotics. *Clin. Microbiol. Infect.* 10(Suppl 4):1–9.

Loc-Carrillo, C., &Abedon, S. T. (2011). Pros and cons of phage therapy. *Bacteriophage*, *1*(2), 111–114. https://doi.org/10.4161/bact.1.2.14590

Luck, A. E., Bhattacharjee, B., Chauhan, A., Ravikumar,V., & Vakilynejad, M. (2022). Utilizing the CRISPR–Cas9 system to create hypervirulent cyanophages as a remedy for harmful algal blooms in saltwater ecosystems. BioTreks, https://biotreks.org/wp-content/uploads/2022/10/e202202.pdf

Mangalea, M. R., & Duerkop, B. A. (2020). Fitness Trade-Offs Resulting from Bacteriophage Resistance Potentiate Synergistic Antibacterial Strategies. Infection and immunity, 88(7), e00926-19. https://doi.org/10.1128/IAI.00926-19

Mayslich, C., Grange, P. A., & Dupin, N. (2021). *Cutibacterium acnes* as an Opportunistic Pathogen: An Update of Its Virulence-Associated Factors. *Microorganisms*, *9*(2), 303. https://doi.org/10.3390/microorganisms9020303

Michael, C. A., Dominey-Howes, D., & Labbate, M. (2014). The antimicrobial resistance crisis: Causes, consequences, and management. *Frontiers in Public Health*, *2*. https://doi.org/10.3389/fpubh.2014.00145

Miller, S.I. (2016). Antibiotic Resistance and Regulation of the Gram-Negative Bacterial Outer Membrane Barrier by Host Innate Immune Molecules. mBio. 7:e01541-16. doi: 10.1128/mBio.01541-16.

Perros, M. (2015). A sustainable model for antibiotics. *Science* 347 1062–1064. 10.1126/science.aaa3048

Pires, D.P., Melo, L.D.R., & Azeredo, J. (2021). Understanding the Complex Phage-Host Interactions in Biofilm Communities. Annu Rev Virol. 8:73–94. doi: 10.1146/annurev-virology-091919-074222

Qin, J., Wu, N., Bao, J., Shi, X., Ou, H., Ye, S., Zhao, W., Wei, Z., Cai, J., Li, L., et al. (2020). Heterogeneous Klebsiella pneumoniae Co-infections Complicate Personalized Bacteriophage Therapy. *Front. Cell. Infect. Microbiol. 10*, 608402.

Qing, N. (2007). China's first doctor of bacteriology–Yu He. *Acta Microbiol. Sin.* 949–950. (In Chinese)

Shariati, A., Dadashi, M., Chegini, Z., van Belkum, A., Mirzaii, M., Khoramrooz, S. S., & Darban-Sarokhalil, D. (2020). The global prevalence of Daptomycin, Tigecycline, Quinupristin/Dalfopristin, and Linezolid-resistant Staphylococcus aureus and coagulase-negative staphylococci strains: a systematic review and meta-analysis. Antimicrobial resistance and infection control, 9(1), 56. https://doi.org/10.1186/s13756-020-00714-9

Skurnik, M., Pajunen, M., & Kiljunen, S. (2007). Biotechnological challenges of phage therapy. *Biotechnol Lett.* 29:995–1003. doi: 10.1007/s10529-007-9346-1.

Silpe, J. E., Duddy, O. P., & Bassler, B. L. (2023). Induction mechanisms and strategies underlying interprophage competition during polylysogeny. PLoS pathogens, 19(5), e1011363. https://doi.org/10.1371/journal.ppat.1011363

Summers, W. C. (2001). Bacteriophage therapy. *Annu. Rev. Microbiol.* 55 437–451. 10.1016/B978-0-12-394438-2.00001-3

Ventola, C. L. (2015). The antibiotic resistance crisis: part 1: causes and threats. *P & T : a peer-reviewed journal for formulary management*, *40*(4), 277–283.Viswanathan, V.K. (2014). Off-label abuse of antibiotics by bacteria. *Gut Microbes.* 2014;5(1):3–4.

World Health Organization (WHO). (2018). *Antimicrobial Resistance.* Available from: https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance