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Yixuan Peng March 29, 2023

# A Study of Population and Evolutionary Dynamics of Temperate and Lytic Phage in *Pseudomonas aeruginosa*

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An abstract of
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## Abstract

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Immunity and resistance are the two major mechanisms that enable bacteria to survive infections with bacteriophage (phage). In the case of resistance, the bacteria are resistant (refractory) to the phage. With immunity, on the other hand, the phage infects the bacteria, but the infection is aborted, and the infecting phage is lost. In the classical interpretation, lysogenic bacteria are immune to the phage-encoded by their prophage. In a recent study, it was predicted and demonstrated with *E. coli* and temperate phage Lambda that under broad conditions selection will favor the evolution of resistant as well as immune lysogens. This prediction was confirmed by testing naturally occurring lysogenic *E. coli* with phage coded for by prophage, all ten studied bore chromosomal mutations resistant for resistance to these phages. In this study, we explore the generality of this finding with fifteen naturally occurring lysogenic strains of Pseudomonas aeruginosa. At one level, the results of this study are consistent with that observed for *E. coli*. However, only five of the naturally occurring *P. aeruginosa* lysogens bore chromosomal mutations for resistance to the phage coded for by their prophage. For the remaining ten, the resistance was coded for by genes borne by the prophage. We consider the implications of these results to our understanding of the population biology, ecology, and evolution of temperate bacteriophage and lysogenic bacteria.

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## Acknowledgments

I would like to thank everyone in the lab, the *Eclf* group. Particularly, I want to thank Brandon Berryhill and Eduardo Roman for providing both theoretical and experimental guidance on this project. I would also appreciate all the suggestions and help from David Goldberg, Joshua Manuel, and the lab manager, Andrew Paul Smith. I also appreciate the support by Kylie Burke and Mason Harvill in the lab. Lastly, I want to thank Dr. Bruce Levin, for giving me the opportunity to join this community and inspiring me in microbiological research.

I would also like to thank all members of my committee for their advice, support, and time devoted to this project. I feel thankful to Dr. Goldberg for providing me with the strains and guidance for future directions and to Dr. Vega for commenting and actively providing feedback on graphs and statistical analysis along the project. Also, I want to thank Dr. George A. O'Toole for providing me with phage strains.

Finally, I would like to thank my parents for their support and funding in me.

This research was funded by Emory University.

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## INTRODUCTION

### Background

Bacteriophages, the viruses that infect bacteria, are perhaps the most intriguing and enigmatic entities in the microbial world. With an estimated $10^{31}$ particles on the planet, they are the most abundant biological agents and are present in virtually all ecosystems, from the ocean floor to the upper atmosphere (*1*). Phages can be classified into two broad categories: lytic and temperate. Lytic phages infect bacterial cells and destroy them as part of their life cycle, while temperate phages can either follow the lytic pathway or integrate their genomes into the host's chromosome, leading to a long-term symbiotic relationship between the phage and the host (*2*).

The study of phages has provided key insights into many aspects of molecular biology, genetics, and gene regulation. For example, phages have been instrumental in elucidating the mechanisms of DNA replication, transcription, and translation. They have also been used as powerful tools for genetic engineering, gene therapy, and biotechnology. Despite their ubiquity and importance, we know surprisingly little about their ecology, evolution, and interactions with their bacterial hosts. We still have much to learn about the ecological and evolutionary roles of phages in bacterial communities. Some of the critical questions that remain unanswered include: How do phages affect the dynamics and structure of bacterial populations? What are the factors that determine the specificity and host range of phages? How do phages contribute to the evolution of bacterial genomes and the emergence of new pathogens? Answering these questions will require a multidisciplinary approach that combines genomics, ecology, and microbiology, among other fields.

With the co-evolutionary arms race between phages and bacteria, bacterial resistance to phages is a common phenomenon that can occur through various mechanisms. In general, the two major defensive mechanisms focus on whether phage entry can be achieved. For immunity, phages inject their genome into the cytoplasm, but, with intracellular defensive mechanisms, replication is aborted, such as restriction-modification systems (*3*), Abi system (*4*), and CRISPR-Cas systems (*5*) For resistance (refractory), the phage cannot bind to the receptor on the bacterial cell surface, fail to inject their genetic material and lyse the cell, such as envelope resistance. By refractory, the distinctive definition places importance on the bacterial cell surface level as the phages cannot bind to the receptor, so the bacteria cell is not susceptible. One of the mechanisms by which bacteria can become resistant to phages is by lysogeny (*6*). During the first infection event, when lysogenic bacteriophages meet a sensitive bacterial population, bacteria can be lysed, which is the most common scenario, form lysogen at an extremely low rate, or select for resistant bacteria. During secondary infection, when lysogenic bacteriophages reinfect their corresponding lysogens, phages are thought to be lost due to immunity due to superinfection exclusion. However, phages can also be maintained during the course of infection via resistance (refractoriness) as suggested. With this evolutionary arms race between bacteria and phages, selection for resistant lysogens rather than immunity will be even more prominent if there are lytic phages that share the same receptor as the temperate phage.

Studies have suggested that naturally occurring lysogenic strains of *E. coli* were resistant (refractory) to the phage coded for by their prophage (*7*). When the prophages are transferred from the wild lysogens to the *E. Coli C* strains, all of the *E. Coli C* lysogens constructed this way

were immune but not resistant (refractory) to the phage coded for by their prophage. This study provided evidence that evolution favors resistance lysogen or refractory. As immunity is not perfect, the incoming genes from the phage, including inhibitory proteins brought by the phages, may disrupt host cell function. However, for resistance(refractoriness), this is a safer mechanism for bacteria to prevent superinfection. With these results, not much has been explored in the *Pseudomonas aeruginosa* system. Previous studies have suggested several types of *Pseudomonas aeruginosa* phages prevent superinfection in various ways. Some of these mechanisms impacted the type IV pilus and O-antigen, while others operated internally within the cell (*8*) (*9*). However, these studies didn't address and provide answers to these questions about the population biology, ecology, and evolution of bacteriophage as well as the contribution of temperate phage to the virulence of pathogenic bacteria. The question of whether naturally occurring lysogens are resistant (refractory) as well as immune to the phages coded for by their prophages is explored in this study.

We address this question by studying *Pseudomonas aeruginosa,* which is an opportunistic pathogen that is known to be intrinsically resistant to multiple antibiotics and a common bacterial infection in cystic fibrosis patients(*10*). It is also commonly found in various environments, including soil, water, and bacterial infections (*11*). This bacterium has a broad host range of bacteriophages (phages), and many strains of *P. aeruginosa* are naturally lysogenic, meaning they carry prophages in their genome. These prophages can provide several benefits to the host bacteria, such as protection against superinfection, increased virulence, and enhanced adaptation to the environment. The prophage can also be a potential source of genetic diversity and can contribute to the emergence of new virulent strains (*12*). It has been

suggested that the presence of prophages may confer resistance to the phages that they encode. We investigated this question by conducting experiments to determine the susceptibility of lysogenic *P. aeruginosa* to their prophage phages. We induced prophage in lysogenic *P. aeruginosa* and evaluated the resulting phage production and the resistance of the host to phage infection. The results of this study have important implications for understanding the dynamics of phage-bacteria interactions in natural environments and may provide insights into the evolution of bacterial resistance to phages.

## RESULTS

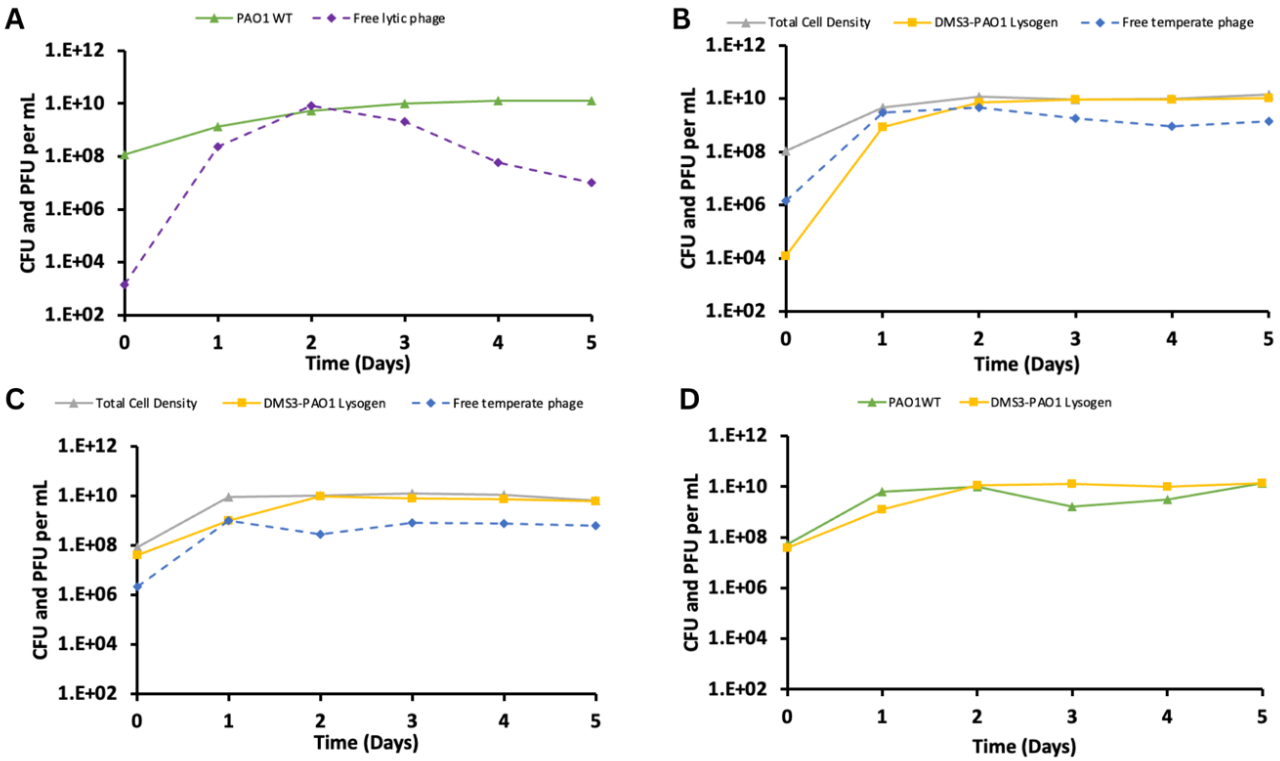


**Figure 1: Population dynamics for DMS3$^{VIR}$ and DMS3$^{GEN}$ in serial passages experiments for PAO1 strains.** Four experiments are presented with PAO1 WT sensitive population and DMS3$^{VIR}$ (A); PAO1 WT sensitive population and DMS3$^{GEN}$(B); DMS3-PAO1 lysogen and DMS3$^{GEN}$(C);

Control experiments of PAO1WT and DMS3-PAO1 lysogens grow independently without phages(D). The grey line with triangle markers represents total cell density. The purple dashed line with diamond markers represents DMS3$^{VIR}$ phage density. One replicate is represented. The yellow line with square markers represents DMS3-PAO1 lysogens. The blue dashed line with diamond markers represents DMS3$^{GEN}$ phage density. The green line with triangle markers represents PAO1 WT.

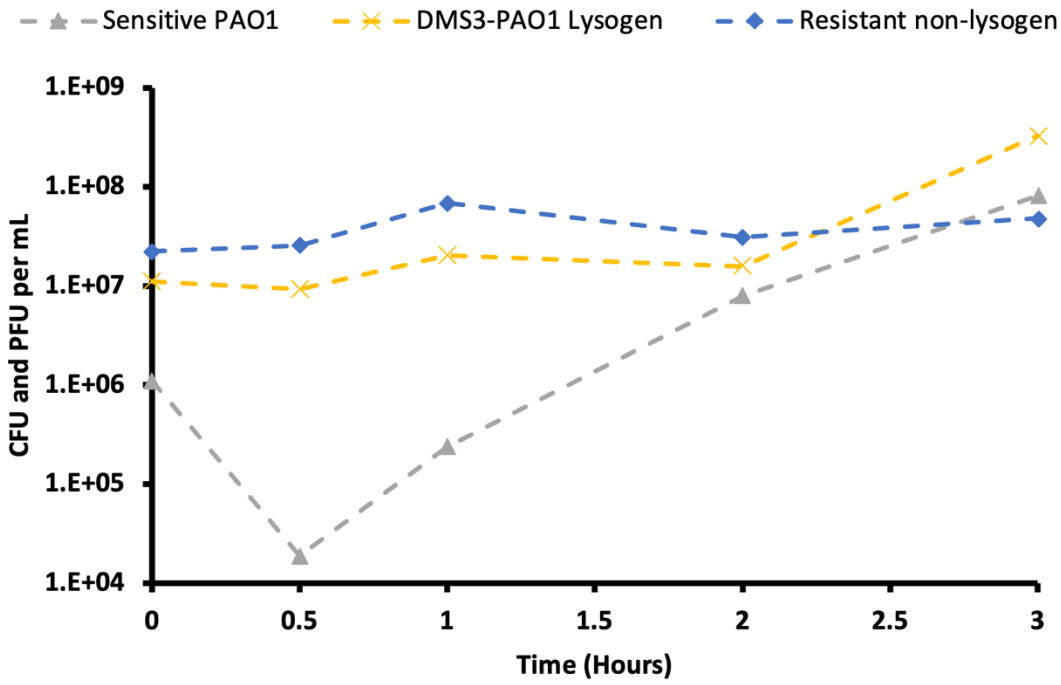


**Figure 2**: **Population dynamics on the absorption of DMS3$^{GEN}$ with three populations for PAO1 strains.** Densities of free phage after 0.5, 1, 2, and 3 hours during infection are graphed, which are the infections with phage DMS3 $^{GEN}$ in three distinct bacterial states: DMS3-sensitive PAO1 WT(A), DMS3-resistant PAO1 WT (B), and DMS3-PAO1 lysogen (C). One replicate is represented

With the modeling and experimental studies done in the *E. coli* study, it can be inferred that, when the sensitive bacterial population is infected with temperate phages, resistant lysogen would be the dominant population and have a higher fitness advantage compared to the susceptible population (*7*). This selection of resistant lysogen is even more prominent in lysogenic bacterial populations with the presence of virulent phages that use the same receptor as the temperate phage. This study uses PAO1 WT as the main bacteria strain with the temperate phage DMS3$^{GEN}$. Resistant non-lysogens were selected with DMS3$^{VIR}$. In the case of DMS3 temperate phages which is a type IV pilus-dependent phage, one study suggests the resistance mechanism is due to the disruption of the type IV pilus function because of prophage(*13*). For the control experiment, as expected, the phages replicated on the sensitive non-lysogens but not the DMS3$^{VIR}$-resistant PAO1 WT(Fig.2). When the sensitive population of bacteria, PAO1 WT, is infected with temperate phage, DMS3, a phage burst is observed, indicating that the free temperate phages are absorbed, bind to the surface receptor of the host cell, inject their genome into the cytoplasm, and then reproduce to create viral particles (Fig. 2A). When resistant lysogens are infected with the temperate phage coded for by their prophage, the host cells are not susceptible, and the temperate phages are maintained in the culture (Fig. 2B). This result is also supported by the long-term serial passage experiment (Fig. 1C). However, when DMS3-PAO1 lysogens are infected with DMS3 temperate phage as secondary infection, it is unexpected to see they are behaving like resistant lysogen, compared to the expectation of superinfection exclusion (Fig. 2C). Also, the results also marked an increase in the density of free phages in a control experiment with DMS3 lysogens infected by temperate DMS3-phage, similar to the *E. coli* study (*7*). In addition, the rate of lysogen

formation between DMS3 temperate phage and sensitive cells is surprisingly high. When a sensitive PAO1 WT- DMS3 sensitive population is initiated with ~$10^8$ density inoculated with ~$10^6$ DMS3$^{GEN}$ phages, DMS3-PAO1 lysogens form almost immediately after plating (Fig. 1B).

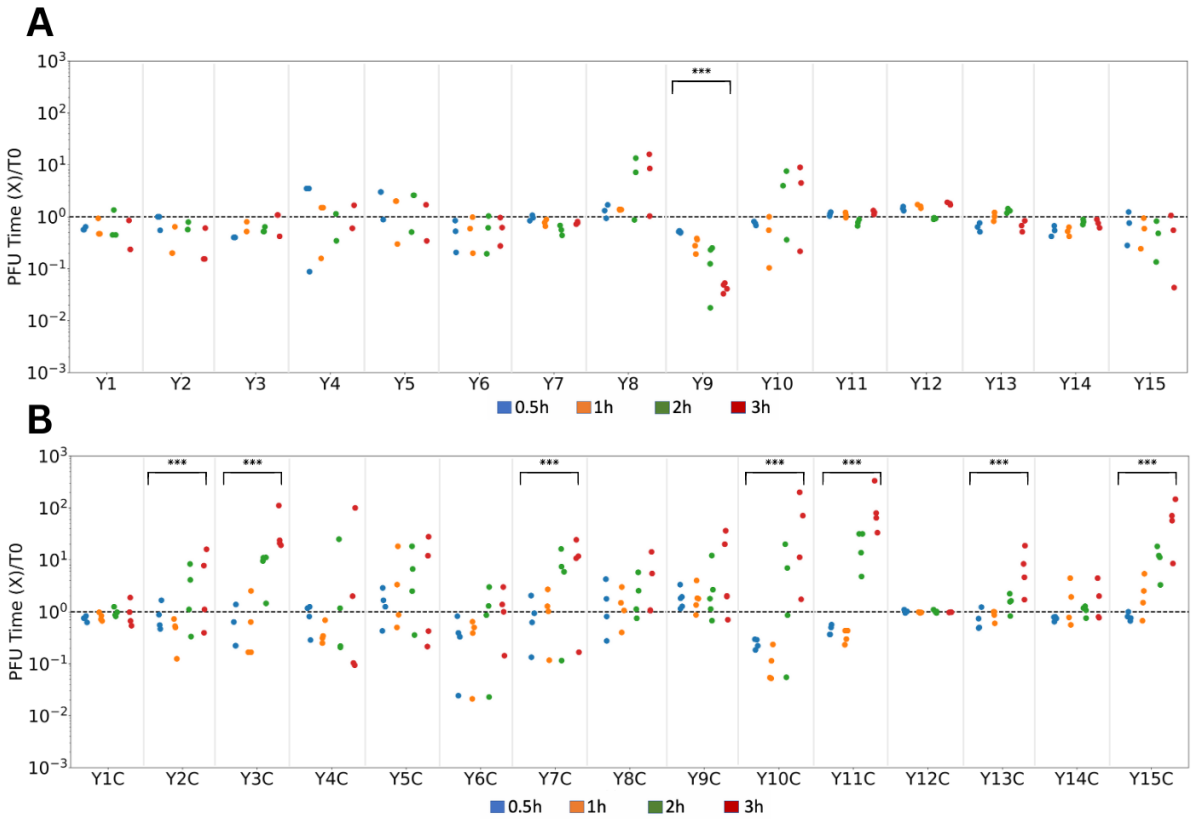


**Figure 3: Absorption of free temperate phage with naturally occurring and *P. aeruginosa* and PAO1 constructed lysogens.** The graph shows the ratios of free phage density after 0.5, 1, 2, and 3 hours compared to the initial density (T0). All data points were presented for four replicates (wild) and three replicates (constructed) of the experiment with boxplots. Two types of lysogens were used in the experiment: (A) naturally occurring lysogens that were infected with low levels of free phages that corresponded to their own lysogenic strain, and (B) lysogens that were constructed from the prophage induced from a corresponding wild lysogen and

infected with low levels of induced wild phages for which they were lysogenic. Linear regressions were conducted for all samples to measure the trend of data between each time point. Three asterisks suggest differences in the distribution of data at a p-value lower than 0.05 (Table S2).

To investigate these predictions, a set of naturally occurring lysogenic *P. aeruginosa* lysogen with different prophages from soil and clinical CF patient was used in the experiment. These wild lysogens were induced to have a single plaque high titer phage lysate, which was then used to infect the lysogens from which they originated. The results showed that most of the samples were consistent with the model and experimental predictions (Fig.3A). Specifically, 14 out of 15 naturally occurring lysogens were refractory to the free phages generated by the induction of their host lysogens; only Y9 suggested immunity (Fig.3A).

Previous works have shown there to be an abundance of prophage-encoded mechanisms which confer a resistance phenotype to their host (*8*). To test the hypothesis that whether the resistance of our naturally occurring lysogens to the phages coded by their prophages is a property of the bacteria or that of the prophage, we infected PAO1 WT with the temperate phages induced from the naturally occurring lysogens. After we transfer the prophage to the wild-type PAO1, we test the absorption of free phage of the constructed lysogen with their corresponding temperate phage. Eleven out of the fifteen wild prophage-bearing lysogens of PAO1, were resistant (refractory) to the free phages coded by their prophage (Fig. 3B). Four of the fifteen constructed lysogens were not refractory to the phage coded for by their prophage (Y3C, Y10C, Y11C, Y15C). Even though Y2C, Y7C, and Y13C suggest

statistically significant on the slope of the best-fitted line with linear regression, they are considered as resistance (refractoriness) as the slope is relatively small compared to other samples (Table S2). This implies the increase may not be significant in scale, so there may not be a burst on the last hour time point. Therefore, a mixture of prophage and cellular chromosomal- resistance (refractories) attribute to the results of this experiment with most of the resistance encoded by the prophage. Interestingly, with the sequencing data, strains Y8 and Y9 may bear the same prophage, yet they have different responses implying a cellular attributed defense (Fig. 3A; Table S1). Again, even though strains Y7, Y12, and Y13 have different distributions of data with statistical significance, we consider them as refractory due to the relatively low magnitude of changes in the log scale. Moreover, when the PAO1-constructed lysogens were infected with a low density of free temperate phages, the four samples that suggest immunity (Y3C, Y10C, Y11C, Y15C) exhibiting high densities of free phages were produced (Fig.3B). Studies on *E. coli* have suggested this is due to the mounting of SOS signals in bacteria caused by external stress and damage on the host DNA, but the molecular mechanism still remains unknown to explain these results with PAO1 constructed lysogen (*7*).

## DISCUSSION

Phages, which are short for bacteriophages, are a type of virus that targets bacteria. They are small in size and diverse, playing a crucial role in regulating bacterial populations in various environments (14). Phages can have two lifestyles: lytic and temperate. Lytic phages invade bacterial cells, exploit their replication machinery, and cause the host cell to lyse, releasing newly generated phages. On the other hand, temperate phages can either follow the lytic cycle or integrate their genetic material into the bacterial genome, becoming dormant.

Bacterial defenses against phage invasions include resistance and immunity mechanisms (6) (15). Temperate phages, which incorporate their genome into the host bacteria, make secondary infections difficult since the previous infection provides the host bacteria with protective mechanisms. Immunity and resistance differ in host defense during secondary infections at the population level. Phages are typically lost during infection due to superinfection exclusion for immunity, whereas envelope resistance prevents phages from binding to the receptor with extracellular mechanisms, preserving the phage population. This study aimed to determine if naturally occurring lysogens are refractory and immune to phages coded for by their prophages. The traditional perspective of immunity does not account for the refractoriness observed in this study.

The study induced a temperate phage from ten naturally occurring lysogens to examine the generality of the refractoriness phenomenon in *E. coli* studies and its model implications (7). All fifteen temperate phages from naturally occurring lysogens could form lysogens with the laboratory strain of PAO1. Contrary to previous experiments and predictions from the model, most of the naturally occurring lysogens were resistant, with some being immune to the phage. This suggests a mixture of defensive mechanisms encoded by their prophage or the cellular chromosome (8). More importantly, these results indicate a large populational phenotypic heterogeneity with significant variation in our samples. Therefore, based on these theoretical and experimental findings, we expect that lysogenic bacteria that occur naturally will also be resistant and immune to the phage that is encoded by their prophage.

The research findings suggest that infections of lysogens with phages, whether they are encoded by their prophage or not, can increase the rate of lysogen induction and production of

free phages by lysogenic populations. This may be due to stress and damage to the DNA of *P. aeruginosa* caused by phage infections. The study also suggests that specific mutations on the receptor or repressor genes may confer resistance to phage infections.

Future studies can focus on the molecular mechanisms of these observations, especially the samples with different responses. Whole genome sequencing and analysis of prophage and chromosomal-attributed refractoriness can help investigate specific genes responsible for resistance. The study also suggests exploring the intracellular mechanisms that contribute to the overall defensive mechanism against phage invasion, including CRISPR/Cas-mediated immunity and how bacteriophages infiltrate biofilm settings to interact with immobile bacteria populations.

In clinical settings, the observation of high lysogen rates in extensively drug-resistant samples may be due to a trade-off between sensitivity to bacteriophages or antibiotics. Overall, the study highlights the importance of understanding the mechanisms of phage resistance and immunity to develop effective strategies to combat bacterial infections.

From an evolutionary perspective, Bacteria appear to favor the temperate lifestyle of bacteriophages, which allows them to escape detection by the host immune system, including the CRISPR-Cas system that recognizes and destroys foreign DNA, such as phages. This is achieved through the integration of their genetic material into the bacterial genome. Additionally, the temperate lifestyle increases the phage's chances of long-term survival in an unstable environment. Lytic phages require new hosts to survive since they die along with their host. In contrast, temperate phages integrate their genetic material into the host genome, enabling them to persist in the host population for more extended periods without causing

immediate cell lysis. This indicates that a temperate life cycle could be more advantageous for bacteriophages than a lytic one. The continued existence of lytic phages in nature poses an intriguing evolutionary question to consider (*18*).

As the theoretical and experimental basis of evidence for this study, the *E. Coli* study suggests that naturally occurring lysogens are likely to be refractory to the phage coded for by their prophages (7). While this study partially supports the hypothesis, it also highlights the importance of testing its generality. The interaction between bacteria and phages is critical in microbiology, affecting disease, evolution, and ecology. While *Escherichia coli* is the most commonly studied bacterial species in host-phage interactions, it is crucial to test the generality of these interactions using other organisms.

The choice of a phage's lifestyle depends on several factors, including the availability of suitable hosts, the presence of other phages, and environmental conditions (19) (20). Different model organisms can have different responses to phage infection. For instance, the population heterogeneity of *Pseudomonas aeruginosa* may be one of the reasons why the hypothesis cannot be readily generalized. Additionally, bacterial strains can vary in their metabolic pathways, stress responses, and virulence factors, which can affect host-phage interactions. Some phages may be better suited to infect certain bacterial strains than others. Therefore, the results of a study on phage-bacteria interactions in one organism may not be applicable to other organisms.

Testing the generality of hypotheses in host-phage interactions using other model systems ensures that the results of a study are relevant to real-world applications. Host-phage interactions play a crucial role in many aspects of human health, including the spread of

antibiotic resistance and the development of phage therapy for bacterial infections. Thus, it is essential to test the generality of hypotheses using a range of bacterial species to ensure that research findings can be generalized to other bacterial pathogens. By testing host-phage interactions in different organisms, researchers can better understand the underlying mechanisms and dynamics of phage-bacteria interactions, leading to more effective phage therapies and management strategies for bacterial infections.

## MATERIALS AND METHODS

### Bacteria

Wild-type (WT) *Pseudomonas aeruginosa* PAO1 was provided by Dr. Joanna B. Goldberg (Emory University). Clinical and soil samples were provided by Dr. Joanna B. Goldberg, Dr. Karen Maxwell (University of Toronto), and Dr. Joseph Bondy -Denomy (University of California, San Francisco). One *Pseudomonas aeruginosa* lysogenic strain was isolated from Lullwater Preserve. All lysogens were made in the Levin Lab and saved at −80°C.

### Phages

DMS3 and DMS3$^{VIR}$ were obtained from George A. O'Toole at Dartmouth College. DMS3$^{GEN}$ was obtained from the Levin lab.

### Cultural Media

Lysogeny Broth (LB) (244620, Difco$^{TM}$): Media that supports the growth of both *P. aeruginosa*

Cetrimide agar: Agar for the selection and differentiation of *Pseudomonas aeruginosa*

Phage plates: Phage plates are prepared with 1g/L Yeast Extract (BD-241750), 10 g/L Bacto-Tryptone (Fischer-BP1421), 8 g/L NaCl (Fischer-S271), 10 g/L Agar (BD-214010), 1g/L Glucose (Sigma-G5767) and 2 mM $CaCl_2$ (Sigma-C5080).

Double-layer soft agar: Double-layer soft agar was prepared with 1 g/L Yeast Extract, 10 g/L Bacto-Tryptone, 8 g/L NaCl, 7 g/L Agar, 1g/L Glucose and 2 mM $CaCl_2$.

Antibiotics:

Gentamicin

Isolation of *Pseudomonas aeruginosa* from soil

10 samples (8 soil samples and 2 water samples) were collected at the Lullwater reserve at Emory University. Samples were collected 15-20 cm below the land surface in 10ml falcon tubes. Each sample was located at least 200 meters far from the other. 5 grams of each sample (5ml for water samples) were inoculated in 25 ml of LB broth and shaken at 37C for an hour. 3 ml of each sample was taken, left to settle for 20 mins, and supernatants serial diluted, and plated 100ul, respectively on Cetrimide agar plates and LB plates. Plates were incubated overnight. The rest of the samples were left to grow overnight. After 24 hours of growth, the samples were settled for 20mins, and the supernatants of ten samples were serially diluted. 100ul of the 1e3 dilutions were plated on LB plates. Original cultures were streaked on Cetrimide agar plates and LB plates and plated (100ul) on Cetrimide agar plates. Suspected colonies (yields green pigmentation) were picked and streaked again on Cetrimide agar and LB plates. After overnight incubation, suspected colonies were picked and streaked again on Cetrimide agar plates.

Phage isolation and Purification

The collection of 35 different genotypic *Pseudomonas aeruginosa* strains and 10 *Pseudomonas aeruginosa* samples from the soil isolates were screened for possible lysogens. The prophage

testing and screening were done with UV lights. A single colony was picked to put into a 10ml flask containing LB broth and incubate at 37°C on a shaker overnight. 300 $\mu$l of an overnight bacterial culture of the strain was tested for lysogeny to 10 ml fresh broth (LB), and incubate at 37 C for 1 h. Cells were then transferred to sterile centrifuge tubes and centrifuged at 6000×g for 10 min at room temperature. Cells were resuspended in 5 ml sterile 0.1M $MgSO_4$. The culture was then transferred to a sterile empty Petri dish. The Petri dish is placed 15 cm from the germicidal short-wave UV lamp for 2 minutes. The suspension was transferred with the $MgSO_4$ to a screw-capped tube containing 5 ml of LB broth. Incubate at 37◦C in the shaker. As the cell was lysed, for approximately 4-6 hours, the culture was centrifuged at 3000×g for 15 min. The supernatant was filtered through .22 filter, tested for titer via traditional plaque assay, and stored at 4◦C. Each lysate was then serially diluted, and 100ul of diluted stock lysate was added with 100ul of the PAO1 WT overnight cultures in soft agar which was then topped on phage plates. The single plaque was then picked with an inoculation loop and enriched with PAO1 WT overnights. Briefly, 100 ul of the overnights were added to a flask containing 10 ml of LB broth. The single plaque was added to the culture after the incubation of 1 hour with 100ul of the 1M $MgCl_2$. As the cell was lysed and phage replicated, for approximately 4-6 hours, the culture was centrifuged at 3000×g for 15 min. The supernatant was filtered through .22 filter, tested for titer via traditional plaque assay, and stored at 4◦C.

Lysogen construction

10 µl of each purified lysate was mixed with 10 µl of PAO1 WT overnight culture in a flask containing 10 ml LB broth. The mixed culture of PAO1 and wild temperate phages were incubated at 37°C with a shaker overnight. A single colony was struck on LB plates for all

mixtures. Individual colonies were inoculated in LB broth and incubated overnights. The resulting overnights were then tested for the presence of phage and confirmation of lysogen formation via UV light. Phage production was then quantified by traditional plaque assays with wild-type (WT) PAO1 as the top agar on phage plates.

Sampling bacterial and phage densities

The densities of bacteria and phages were assessed by diluting them in 0.85% saline and plating. The total bacterial density was estimated on Lysogeny Broth (LB) IN hard (1.6%) agar plates. In competitions that involved multiple bacterial and phage populations, diluted samples were plated on LB hard (1.6%) agar plates with gentamicin (4 ug/mL) to differentiate between sensitive and lysogenic bacteria. To determine the densities of free phage, the suspensions were treated with chloroform, diluted, and then plated at different concentrations on LB agar plates covered with 4mL of LB soft (0.7%) agar and 0.1 mL of overnight LB-grown cultures (about $6\times10^8$) of PAO1 WT.

Testing for Resistance

To assess the bacteria's ability to resist $DMS3^{VIR}$, they were streaked on LB hard (1.6%) agar plates. A line consisting of 200 µL of a $DMS3^{Vir}$ lysate with a concentration exceeding $10^8$ pfu/mL was drawn on the plates. If the bacteria formed a continuous line on the agar, it was interpreted as an indication of their resistance. If the line was broken, it was seen as a sign of their sensitivity to $DMS3^{Vir}$.

Testing for whether the bacteria are refractory or immune to the phage

In this experiment, a large number of bacteria were exposed to a low multiplicity of infection (0.01-0.1) with phage in a 10 ml volume of LB broth. The phage density was serially diluted and measured at T=0, T=0.5, 1.0, 2.0, and 3.0 hours to assess its impact on the bacteria with chloroform to remove bacteria in the samples at each time point. If the density of the bacteria remained relatively unchanged, they were categorized as refractory (resistant) to the phage. Conversely, if the phage density decreased or increased in later samples, the bacteria were deemed to be immune.

Serial transfer experiments

50 ml Erlenmeyer flasks were filled with 10 ml of LB broth and maintained at 37°C with vigorous shaking. The serial transfer cultures were typically initiated by diluting 10-mL overnight cultures grown from individual colonies by a factor of 1:100. Phages were added to the cultures at densities specified in the figures. After each transfer, a volume of 100µl from the cultures was introduced into flasks containing fresh medium diluted 1:100, and samples of 0.1 mL was obtained to measure the densities of bacteria and phages.

Sequencing and analyses

Phage DNA was extracted using Invitrogen's (California, USA) PureLink Viral RNA/DNA extraction kit (Cat# 12280-050) using the manufacturer's protocol and bacterial DNA was extracted using ZYMO's research quick-DNA HMW MagBead Kit (Cat# D6060 ) using the manufacturer's protocol. The extracted DNA was quantified on ThermoFisher's NanoDrop OneC microvolume spectrophotometer (Cat# ND-ONE-W). Samples were sent to SeqCoast for

sequencing. Briefly, samples were prepared for whole genome sequencing using an Illumina DNA Prep tagmentation kit and unique dual indexes. Sequencing was performed on the Illumina NextSeq2000 platform using a 300 cycle flow cell kit to produce 2x150bp paired reads. Analysis of FASTAq files received from the Microbial Genome Sequencing Center was analyzed using Geneious Prime version 2022.0.1.

Statistical Methods Statistical analysis of significance was carried out by linear regressions across different time points and calculations were performed using Python.

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Data and materials availability

The Berkeley Madonna program used for the simulations is available at www.eclf.net .

The sequence data shown have been deposited at Genbank (NCBI, Bethesda, Maryland, USA).

Supplementary Materials

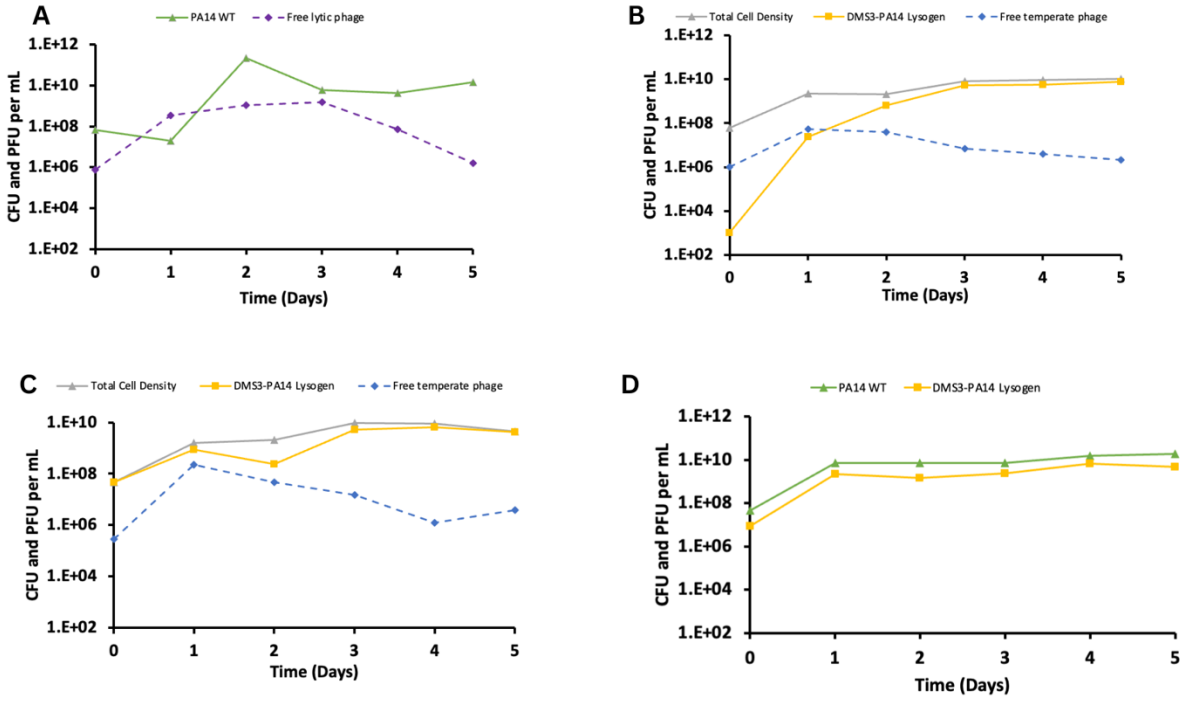
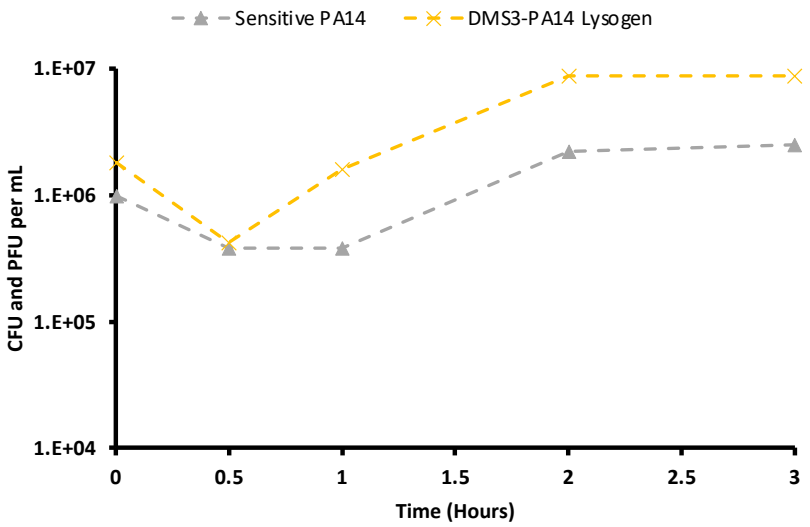



**Figure S1. Absorption of DMS3$^{VIR}$ and DMS3$^{GEN}$ in serial passages experiments for PA14 strains**. Infections with PA14 WT sensitive population and DMS3$^{VIR}$ (A); PA14 WT sensitive population and DMS3$^{GEN}$(B); DMS3-PA14 lysogen and DMS3$^{GEN}$(C); Control experiments of PA14WT and DMS3-PA14 lysogens grow independently without phages(D). The grey line with triangle markers represents total cell density. The purple line with diamond markers represents DMS3$^{VIR}$ phage density. The yellow line with square markers represents DMS3-PA14 lysogens. The blue line with diamond markers represents DMS3$^{GEN}$ phage density. The green line with triangle markers represents PA14 WT.

**Figure S2. Population dynamics on the absorption of DMS3$^{GEN}$ with three populations for PAO1 strains.** Densities of free phage after 0.5, 1, 2, and 3 hours during infection are graphed, which shows the infections with phage DMS3 in three distinct bacterial states: DMS3-sensitive PA14 WT (A), and DMS3-PA14 WT lysogen (B)




Supplemental Table S1. Wild phage genomes: BlAST results, the percent identity on BLAST results (percent identity between top candidate on BLAST and partial phage sequence), and accession numbers are presented.

| Wild lysogen | Prophage(s) BLAST | Percent identity | Constructed Lysogen | GenBank accession numbers |
|---|---|---|---|---|
| Y1 | Streptomyces phage Lika | 83.06% | Y1C | OQ572403 |
| Y2 | Caulobacter phage BL47 | 84.31% | Y2C | OQ572404 |
| Y3 | Caulobacter phage CcrColossus | 94.44% | Y3C | OQ572405 |
| Y4 | Caudoviricetes sp. | 72.72% | Y4C | OQ572406 |
| Y5 | Pseudomonas phage YMC11/11/R1836 | 88.14% | Y5C | OQ572407 |
| Y6 | Caudoviricetes sp. isolate ct9271 | 69.76% | Y6C | OQ572408 |
| Y7 | Caudoviricetes sp. ctX1E6 | 69.04% | Y7C | OQ572409 |
| Y8 | Siphoviridae sp. ctnLs3 | 99.95% | Y8C | OQ572410 |
| Y9 | Siphoviridae sp. ctnLs3 | 99% | Y9C | OQ572411 |
| Y10 | Pseudomonas phage pf8_ST274-AUS411 | 100.00% | Y10C | OQ572412 |
| Y11 | Pseudomonas phage vB_PaeP_YA3 | 99.12% | Y11C | OQ572413 |
| Y12 | Pseudomonas phage UMP151 | 95.17% | Y12C | OQ572414 |
| Y13 | Pseudomonas phage vB_Pae_CF78a | 100.00% | Y13C | OQ572415 |
| Y14 | Pseudomonas phage ZCPA1 | 97.11% | Y14C | OQ572416 |
| Y15 | Pseudomonas phage vB_Pae_BR313b | 92.86% | Y15C | OQ572417 |

Supplemental Table S2. Linear Regression Results: P-values and slopes for statistically significant results

| Sample | P-Value | Slope |
|---|---|---|
| Y9 | 0.000 | -2.02 |
| Y2C | 0.044 | 1.93 |
| Y3C | 0.190 | 13.7 |
| Y10C | 0.052 | 21.9 |
| Y11C | 0.024 | 40.2 |
| Y13C | 0.018 | 2.36 |
| Y15C | 0.008 | 21.9 |