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Carissa Hutchison April 15, 2019

Environmental Effects on Lambda Lysogeny and Spiteful Attack

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Abstract

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Lysogeny is one of two life paths that a lambda phage may take when entering a susceptible cell. The probability of lysogeny varies with environmental factors; such as the density of susceptibles, chemical factors, and number of phage present. Additionally, lysogeny appears to have many benefits for the cell and the phage. One potential benefit of lysogeny is the capability of a lysogen to use spiteful attack, where the lysogen induces so that the phage can kill off any competitors and allows the lysogen population to have control over a resource rich area. The current study examined the effects of environmental factors on lambda lysogeny and spiteful attack in *Escherichia coli*. Specifically, the effect of nutrition and changing the initial population ratios between lysogenic and sensitive bacteria. First, parameters for the bacteria and the bacteriophages were determined in each of the environmental conditions. Then, competition assays were run between one lysogenic strain and one sensitive strain for three generations. For 1:1 assay using the MG1655-λ_{kan}, the lysogenic bacteria outcompeted the sensitive bacteria in the LB and S-medium + 0.1% NH_4Cl + 0.1% Glucose. Future studies should look at the impact varying the ratio of sensitive bacteria to the MG1655-λ_{kan} and a slower growing lysogen than the MG1655-λ_{kan}, and the impact of spatial structure on competition.

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Introduction

Bacteriophages (phages) are viruses that infect and typically kill bacteria. A temperate phage can undergo two general life cycles—lytic or lysogenic. During the lytic cycle, the phage infects the bacteria and replicates rapidly until the phage progeny burst out of the cell. The lysogenic pathway involves the phage integrating itself into the bacterial DNA. The integrated phage (prophage) is maintained in the bacterial DNA via a viral repressor that prevents the phage from entering the lytic cycle. When the repressor is cleaved, the phage then goes through the lytic cycle, killing the host. Although temperate phage therefore give up fitness temporarily by integrating into the bacterial DNA, there is considerable theoretical and experimental evidence indicating that lysogeny can increase the overall fitness of a phage under certain conditions. However, the factors supporting lysogeny from the standpoint of host fitness are less well understood.

Lysogeny is selected for in phage when the prophage is expected to have higher fitness than lytic phage. When probability of horizontal transmission is low, for example when there is a low density of susceptible hosts, the expected fitness of a prophage is higher than that of a virulent phage, and temperate phages will favor lysogeny rather than the lytic life cycle (1). This is because, in environments where horizontal transmission of phage is unlikely, vertical transmission in the host genome is expected to result in higher numbers of "progeny", copies of the phage in the total population, than horizontal transmission via lysis. Lysogeny is therefore common in the gastrointestinal tract due to the low density of susceptible hosts (2).

Lysogeny is also favored in environments where the bacterial host growth is slow (3), such as nutritionally deficient environments (4). This can reflect a "bet-hedging" style waiting

game, where the bacterial host is expected to eventually enter an environment where optimal growth (5) is achieved and the phage can then undergo lysis and release a higher number of phage progeny.

Integration of phage into the host genome can prove to be a one-way process for the phage. Integration can also lead to mutations in the prophage which can impact its ability to replicate and kill its bacterial host (6). These non-lytic "cryptic" prophage are frequently retained by the host and can provide benefits that increase the survival of their hosts in adverse environments (7).

This suggests that the benefits provided by an integrated prophage can outweigh the costs of lysogeny for the host. Carriage of an active prophage can be costly, as re-activation of the prophage will kill the host. The costs of lysogeny are expected to be high in gut-associated communities (2), where lysogeny is also common (8-9). It is reasonable to suppose that the benefits of prophage carriage outweigh the costs for the host, or lysogens would be outcompeted in this environment; however, it is not well understood which of the potential benefits of lysogeny contribute strongly to its maintenance in natural populations of bacteria.

One potential benefit of lysogeny is transduction, transfer of beneficial genes (e.g. antibiotic resistance cassettes) from one host to another via genome integration of temperate phage. Transduction in general, and transfer of drug resistance genes specifically, can occur at high frequency in the wild (10), even between different bacteria species (11). Furthermore, a high number of antibiotic resistance genes can be carried within the phage population (12); however, not all studies have reached the same conclusion (13). The actual contribution of

phage-mediated transduction to propagation of antibiotic resistance genes is a topic of considerable debate.

Lysogenic bacteria may gain immunity to the specific prophage and related phages (6). While this does not always occur, phage in the lysogenic life cycle can protect their bacterium from infection via related lytic phage if an epidemic of the lytic phage were to occur in the environment. Generally, however, lysogenic bacteria will be immune to attack by virions of the prophage that they carry.

Additionally, lysogeny can change the growth rate of the host bacterium. In some cases, prophage carriage can increase the growth rate of a microbe, allowing it to out-compete non-lysogens in a shared environment (14-15). When lysogeny occurs in nutritionally- starved bacteria, the prophage may slow down the growth rate of the bacteria, allowing it to survive when few energy sources are available (1).

Spiteful attack is a potential benefit of a bacterium becoming a lysogen. A lysogenic bacterium may re-activate phage to kill competing strains, freeing up niche space for the lysogen to occupy (6). Spite is an ecological term that encompasses an action that an organism takes that decreases its own fitness and the fitness of its competitors. Generally, spiteful behaviors are associated with kin selection, where an individual sacrifices their immediate fitness (here, by dying during lysis) to increase the fitness of close relatives (16). Spiteful attack via temperate phage can allow for the lysogenic bacterial lineage to take over and control resource-rich areas; however, the sensitive bacteria may rapidly become lysogens themselves (17-18) and lose susceptibility to the phage. Thus, the lysogenic strain's ability to invade and take over a resource-rich area would be decreased. The overall efficacy of spiteful attack via lysogen

reactivation should therefore depend on the rates of the interactions between sensitive bacteria and phage, the fitness costs of prophage induction, and the competitive interactions between sensitive cells and the original lysogens.

The current study examined multiple factors that could impact the fitness of the lysogen and potentially the benefits of spiteful attack. Specifically, we sought to determine which environmental factors, if any, can impact the potential efficacy of spiteful attack, using the well-studied *Escherichia coli* λ phage system. Competitions between *E. coli* lysogen and sensitive strains were run in nutritionally different media and at different initial ratios of lysogen to sensitive cells. Prior to the competitions, parameters of the bacteria and the temperate bacteriophage, λ_{kan}, were determined in each of the different media. Parameters tested for the bacteria included growth rate, lag time, and carrying capacity in the different media. For the phage, parameters included latent period, burst size, adsorption rate, lysogen probability, and lysogen induction rate.

The population dynamics were observed over three serial passages (approximately 40 generations) to determine how a lysogenic *E. coli* competed with a non-lysogenic strain of *E coli*, and to see if/when spiteful attack was used and under which conditions it was beneficial.

Methods

Bacterial Strains and Media

The study used five *E. coli* strains with different antibiotic resistances and sensitivities to λ_{km} (Table 1). Differences in antibiotic resistance were used to allow selective plating of lysogens, sensitive cells, and newly-formed lysogens on agar plates for enumeration of sub-populations during competition. All bacteria strains were used, except when noted.

Table 1 • Properties of Each *E. coli* Strain

BACTERIA	LINEAGE	SENSITIVITY TO PHAGE	ANTIBIOTIC RESISTANCE	OTHER	BASE STRAIN INFORMATION	ABBREVIATION
MG1655	K12	Lysogen	Kanamycin (from phage)	$+\lambda_{cI857}$	Wildtype. Faster grower.	MKM
DH5α	K12	Lysogen	Kanamycin (from phage)	$+\lambda_{cI857}$	*RecA* deficient. Slower grower	DKM
EMG2	K12	Tolerant	Spectinomycin	mCherry	Wildtype	EMG
DH5α	K12	Sensitive	Gentamycin	Green Fluorescent Protein	*RecA* deficient. Slower grower	DGM
MG1655	K12	Sensitive	Gentamycin	Green Fluorescent Protein	Wildtype. Faster grower.	MGM
MG1655	K12	Sensitive	N/A	N/A	Wildtype. Faster grower.	MG1655

Three media with varying nutritional levels were used in this study. Two S-medium based media and LB were used (Table 2). S-medium is a minimal phosphate buffered medium supplemented with trace minerals which is used for liquid culture of *Caenorhabditis elegans* (19); this media was selected to allow future work with this phage-bacteria system using *C. elegans* as a host. All the experiments were run in each media except for when noted.

Table 2 • Formulas for Each Medium Used

MEDIUM	BASE MEDIUM FORMULA (1L)	ADDITIONS	ABBREVIATIONS	STERILIZATION METHOD
LB	10g bacterial tryptone 10g NaCl 5g yeast extract 1L of DD_2O 15g bacterial agar (for plates)	N/A	LB	Autoclave
S-MEDIUM	S buffer 5.85g NaCl 6g KH_2PO_4 1g K_2HPO_4 1L DD_2O 10mL trace minerals 10mL 1M K-citrate buffer (pH 6.0) 1mL cholesterol 3mL 1M $MgSO_4$ 3mL 1M $CaCl_2$	0.1% NH_4Cl 0.1% Glucose	GLC	Filter Sterilized
		0.1% NH_4Cl 0.1% Glycerol	GLY	

Growth Parameters

Growth curves of each bacterial strain were performed in all three media. Each *E. coli* strain was inoculated 1:100 into 150µL of each media in a 96-well transparent multi-well plates, covered with Breathe-Easy gas permeable membrane (Research Products International) then had the OD recorded every fifteen minutes for 24 hours at 37ºC with shaking on a BioTek HTX multimode plate reader. Growth rates and lag times for each strain x media combination were then calculated. Lag time was calculated by averaging and taking the standard deviation of the first 10 time points (5 time points in LB cultures) to account for machine error. The standard deviation was multiplied by 10 and added to the average. The lag time was the first time point greater than that sum. Growth rates were calculated by taking the slope of the log-transformed data during exponential growth and averaging the rates for three replicates.

MIC Determination

The MICs of each strain were measured in each media for each antibiotic used in the study – kanamycin, spectinomycin, and gentamycin. Each strain was inoculated 1:100 into media containing different levels of each antibiotic, incubated at 37ºC with shaking at 298 rpm for 24 hours, and observed for turbidity to determine MIC. The MICs of each bacterial strain determined the concentration of antibiotics used for selective LB plates for spot plating and to ensure there was no cross-resistance between strains (Table 3).

Table 3 • MICs of Each *E. coli* Strain to the Antibiotics Used for Selective Plating in Each Media. LB (A). GLC (B). GLY (C).

BACTERIA	GENTAMYCIN (μg/mL)	SPECTINOMYCIN (μg/mL)	KANAMYCIN (μg/mL)
EMG	10	>250	10
DKM	2	50	>250
MKM	10	100	>250
MGM	250	50	50
DGM	250	50	10

A

BACTERIA	GENTAMYCIN (μg/mL)	SPECTINOMYCIN (μg/mL)	KANAMYCIN (μg/mL)
EMG	50	>250	0.4
DKM	2	50	>500
MKM	0.4	50	>500
MGM	250	50	10
DGM	250	100	50

B

BACTERIA	GENTAMYCIN (μg/mL)	SPECTINOMYCIN (μg/mL)	KANAMYCIN (μg/mL)
EMG	50	>250	0.4
DKM	50	50	>500
MKM	50	10	500
MGM	>250	100	50
DGM	250	10	50

C

Selective Plating

Selective LB plates were made using MIC data. Plates were made with a kanamycin concentration at `100 µg/mL, gentamycin concentration at 50 µg/mL, and spectinomycin concentration at 100 µg/mL.

Phage Plating

Phage plates were made using phage agar plates and phage top agar. Typically, 100 µL of a bacterial strain and the diluted phage solution were added to 1 mL of top agar then poured onto and spread over one phage agar plate.

Table 4• Formulas of phage agar used for phage plating

MEDIUM	FORMULA (1L)	STERILIZATION
PHAGE AGAR	10g Bacterial Tryptone	Autoclave
	8g NaCl	
	1g Glucose	
	1g Yeast Extract	
	1L of DD_20	
	10g Bacterial Agar	
	2mL of $CaCl_2$ (Added after autoclaving)	
PHAGE TOP AGAR	10g Bacterial Tryptone	Autoclave
	8g NaCl	
	1g Glucose	
	1g Yeast Extract	
	1L of DD_20	
	7g Bacterial Agar	
	2mL of $CaCl_2$ (Added after autoclaving)	

Bacteriophage parameter estimation

Parameters of the bacteriophage λ_{kan} were also determined. The latent period/burst size and adsorption rate, lysogen probability, and lysogen induction rate for bacteriophage λ were measured in all the media. Overnight cultures incubated at 37ºC with shaking at 200 rpm of phage-sensitive MG1655 were re-inoculated and incubated in the same conditions into fresh media an hour (LB) to four hours (GLC and GLY) prior to running the assays, dependent on the media and the growth curve of MG1655 in the media. For all the phage assays, phage were added to the bacterial culture to achieve a final concentration of $1x10^6$ PFU/mL.

Both the latent period/burst (equation 1) and adsorption rate (equation 2) assay involved taking samples from cultures that were incubated at 37ºC with shaking at 298 rpm. The cultures were then pre-inoculated 4 hours before the assay and then λ_{kan} was added to MG1655 20 minutes before being inoculated into 1:100 into fresh media. Samples of 100 µL were taken and chloroformed to kill any bacteria every 15 minutes for three and half hours. Then, three dilutions ($1x10^{-1}$, $1x10^{-2}$ and $1x10^{-3}$) were plated on phage plates. Samples at time 0 were filtered and plated on phage plates to determine the free phage count at time 0. Plates were incubated at 37ºC for 24 hours before enumeration of plaques or CFUs. For the adsorption rate assay, the phage were allowed to adsorb for 30 minutes before samples were taken every 15 minutes to determine CFU/mL (bead plated on LB) and PFU/mL (phage plated).

$$Burst\ size = \frac{(Average\ Free\ Phage\ High)}{(Average\ Free\ Phage\ Low)} \quad (Eq. 1)$$

$$Adsorption\ Rate = -\frac{\frac{change\ in\ phage}{change\ in\ time}}{number\ of\ bacteria * number\ of\ free\ phage} \quad (Eq. 2)$$

The probability of lysogen formation and the lysogen induction rate were both run. Overnight cultures were inoculated 1:100 into 1mL of fresh media in glass tubes and allowed to grow for 2 hours (LB) or 6 hours (S medium). Phage were added to each culture at $1x10^6$, $1x10^7$ and $1x10^8$ PFU/mL and allowed to adsorb for 30 minutes at 37ºC with shaking. The culture was then centrifuged for 1 minutes at 9000 rpm in a tabletop centrifuge (Eppendorf 5424R), washed 4x to remove free phage, and incubated in fresh LB medium with shaking at 37°C for 20 minutes to allow phage integration and gene expression. Total bacterial counts were determined at the start of outgrowth through plating on non-selective media, and lysogens were sampled after outgrowth at $1x10^0$ and $1x10^{-1}$ on LB+Kan50 plates via bead plating. The initial supernatant was used for phage quantification; PFU counts were sampled at the appropriate dilutions by plating on sensitive *E. coli* MG1655 lawns on phage plates.

Induction timing and rate were determined by selecting lysogens formed during the lysogen probability assay. For determining timing of induction, lysogens were grown to stationary phase in LB + Kan50 for selection, then diluted 1:100 into fresh buffer in Eppendorf tubes, washed 4X to remove free phage, then resuspended into 10 mL of fresh media and incubated at 37ºC with shaking at 200 rpm. At 0, 1, 2, 4, 6, and 8 hours, 500 µL aliquots were taken from each culture; 10 µL of these cultures were used for dilution plating on LB+Kan to determine CFU/mL, and the remainder was chloroformed to release sorbed phage and assayed for PFU counts as previously described. Induction rate was then determined using a similar protocol, with timing determined by the results of the previous assay. At the indicated time points, CFU/mL counts were determined as before, and phage counts were determined from chloroformed samples at $1x10^{-2}$ and $1x10^{-3}$ on lawns of sensitive MG1655.

Calculations were run to determine the parameters for which each experiment tested. Burst size was calculated by equation 1. The latent period was the time before the high burst occurred. Adsorption rate was calculated by using equation 2 where N is the number of bacteria, P is the free phage count at T_0, and the $\delta P/\delta t$ is the slope of the trend line. Lysogen probability was calculated by using equation 3 and induction rate was calculated using equation 4.

$$Lysogeny\ probability = \frac{Number\ of\ lysogens\ formed}{Total\ number\ of\ phage\ plaques}\ (Eq.3)$$

$$Induction\ rate = \left(\frac{Adsorption\ Rate}{Burst\ size}\right) * Free\ phage\ count\ (Eq.4)$$

Competition Assays

Competition assays were performed in the three media. For the competition, all *E. coli* strains were used except for MG1655. Each bacterial strain was cultured overnight in 1 mL LB at 37ºC, then were washed two times in 1 mL in PBS to remove any metabolites and/or free phage that may be present from the overnight culture before inoculation at 1:100 into fresh media. A 96-well plate was set up with three columns of each media. Each well contained 150 µL of media and were inoculated with a further 1:100 dilution of one lysogen and one sensitive strain (final dilution $1:10^4$ from stationary phase). A Breathe-Easy gas permeable membrane (Research Products International) covered the plate before the plate was incubated at 37ºC with shaking at 200 rpm for 48 hours. After 48 hours, the populations were serially diluted; and 10 µL of the 10^5, 10^6, and 10^7 dilutions were plated onto selective LB plates – containing either gentamycin, spectinomycin, kanamycin or a combination of the drug selective for the sensitive strain + kanamycin (to select for newly formed lysogens). Levels of antibiotics were determined from the MICs of the lysogen compared to the respective sensitive strains. The population was

then inoculated 1:100 into fresh media to propagate the next generation. Each competition assay was run for three rounds of re-inoculation. The selective plates were incubated at 37ºC for 24 hours before colonies were counted.

Results

Bacterial Parameters

Comparisons of growth rates and lag times were made between sensitive and lysogenic bacteria that shared the same base strain, and within the same strain in the different media. In all cases, the growth rates for each *E. coli* strain decreased in both S-media relative to LB, while the lag times increased (Figure 1). For both MG1655 and DH5α strains, the lysogenic strain grew faster than the sensitive strain (Figure 1).

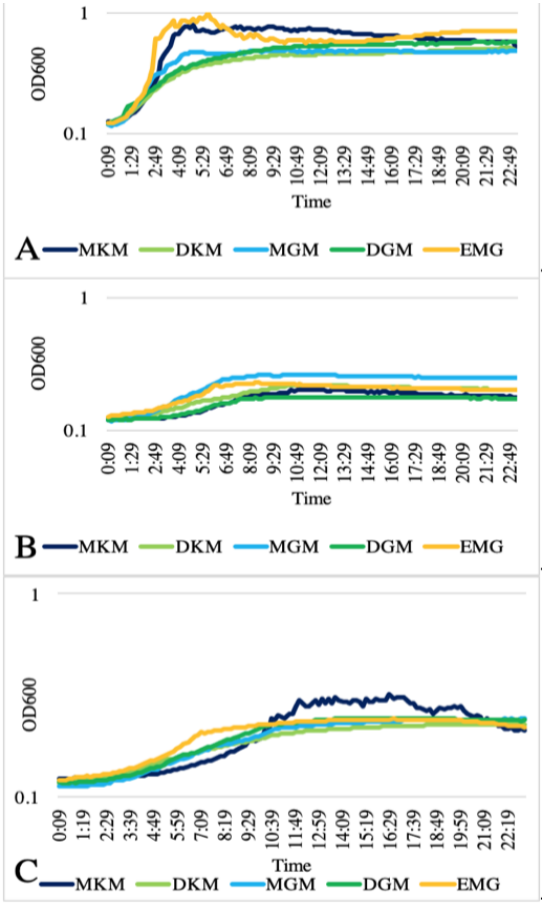

Figure 1• Growth curves for bacteria used in the study and relevant parameter estimates in each media. LB (A). GLC (B). GLY(C). Growth rates are in units of hr^{-1}; lag times are in hours. *E. coli* strains are: EMG, *EMG2*; DGM, DH5α-GmR-GFP; MGM; MG1655-GmR-GFP; DKM, DH5α+λ_{Km}; MKM, MG1655+ λ_{Km}. Note that "KM" strains are lysogens.

Table 5• Quantifications of growth rates and lag times for the bacterial strains used during the study. Growth rates ± standard deviation. Note that "KM" strains are lysogens.

BACTERIA	MEDIA					
	LB		*GLC*		*GLY*	
	Growth Rate (hr^-1)	Lag Time (hr)	Growth Rate (hr^-1)	Lag Time (hr)	Growth Rate (hr^-1)	Lag Time (hr)
EMG2-SPR-MCHERRY	3.66E-01±0.15	1.65	2.00E-02±0.0008	3.57	2.00E-02±0.003	4.15
DH5A-GMR-YFP	6.00E-02±0.01	2.95	1.00E-02	4.20	1.00E-02±0.0005	3.54
MG1655-GMR-YFP	1.20E-01±0.002	1.32	3.00E-02±0.002	3.00	1.00E-02	4.00
DH5A-KMR	6.00E-02±0.005	2.20	1.00E-02±0.003	3.50	1.00E-02±0.0009	4.03
MG1655-KMR	3.40E-01±0.2	1.65	1.00E-02±0.0005	4.50	1.00E-02±0.001	2.93

Bacteriophage Parameters

Burst size and latent period assays were run in all three media using sensitive MG1655 and free λ. The only medium in which a burst size could be calculated was in LB. The burst size of lambda phage in GLC and GLY could not be calculated (Figure 2).

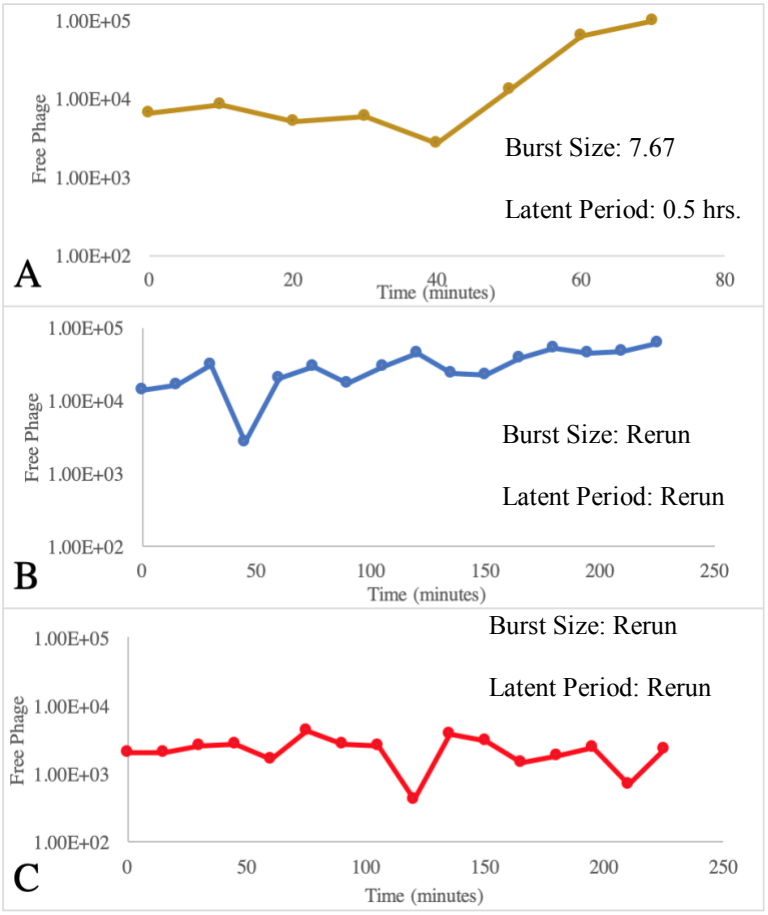

Figure 2• Free phage count over time in LB (A), GLC(B), and GLY(C). X-axes are on different scales because burst size/latent period experiments in the S-media were run for longer periods to give the λ phage time to replicate and burst in the slower growing cells.

Adsorption rate assays were also run in all three media. It was noted generally that lambda phage in GLY has the highest adsorption rate out of the three media (Figure 3). However, all three adsorption rates were all relatively similar, differing by at most a factor of two in the point estimates (Figure 3). Given the inevitable technical noise in these assays, it is plausible that there is no real difference in adsorption rates of phage λ to its host in these three media

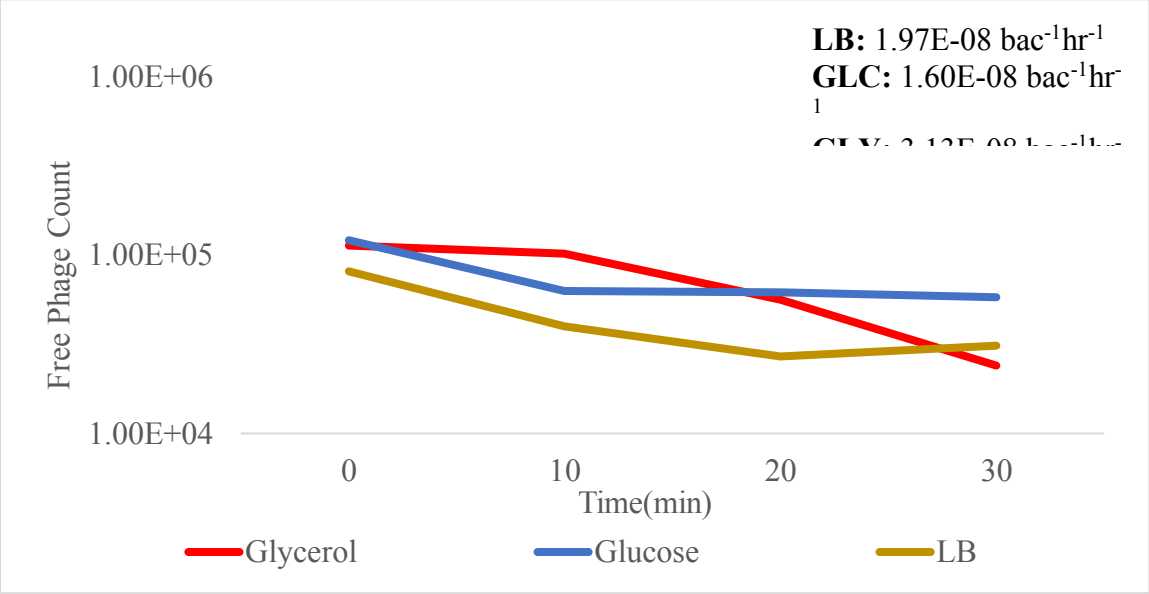

Figure 3• Adsorption of free phage. Adsorption was measured over a thirty-minute interval in LB, GLC, and GLY

Competition Assays

The initial competition assays were run in each of the three media between MKM and three sensitive strains: DH5α-GmR-GFP (DGM), MG1655-GmR-GFP (MGM), and EMG2. The lysogenic bacteria and sensitive strain were initially inoculated at a 1:1 ratio. No growth was noted in the GLY media for any combination. EMG2 outcompeted the lysogen in both LB and GLC, consistent with the observation that this strain is not susceptible to the λ phage. In the

remaining two competitions (MGM and DGM), the sensitive bacteria were outcompeted by the lysogen and were below the limit of detection ($5.00x10^6$ cells) in both LB and GLC. (Figure 4).

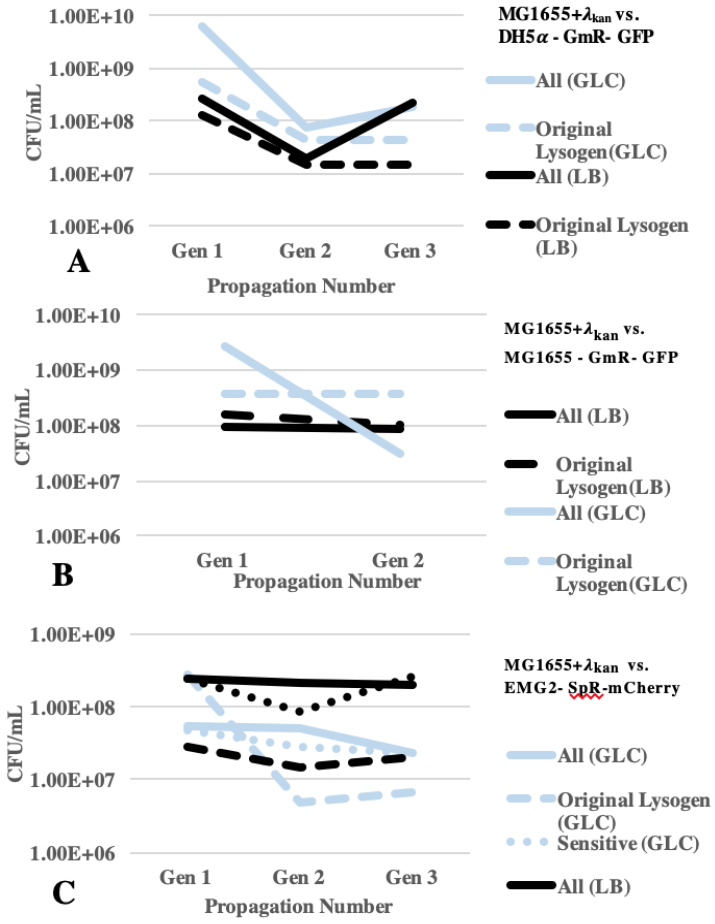

Figure 4• Competition assays between MG1655 + λ_{Kan} and sensitive bacterial strains DH5α+*GmR*+ GFP (A). MG1655+*GmR*+GFP (B). EMG2+*SpR*+mCherry (C), initial 1:1 ratio of sensitive cells to lysogens. Solid lines are in LB. Dotted lines are in GLC. No growth was seen in GLY.

Discussion

Spiteful behavior has been seen in *Escherichia coli* with colicins. An *E. coli* cell can release a toxin which can, but does not always require the death of the cell. These colicins will then kill any bacteria that does not carry the genes for cognate antidote systems, which are encoded in the same locus as the colicin production genes (16). Thus, a population of *E. coli* with

colicins can use those colicins to kill off competitors in an environment. Bacteriophages may allow for a similar effect; but unlike colicins that need to be produced by the bacteria, bacteriophages are able to replicate themselves. The current study determined the effect of environmental factors on λ lysogeny and spiteful attack, specifically in different nutritional environments and different initial population ratios. This effect would be mediated through changes in the parameters for the bacteria and the bacteriophages. Overall, it was seen that the parameter estimates for the bacteria change when put into different nutritional media as the growth rates decreased and lag times increased from the LB to the two S-media.

For the bacteriophages, many parameter estimates could not be estimated yet. Concerns about the physiological state of the bacteria in each media while the experiments were being run were raised so the experiments will be rerun. Specifically, differences in lag time and growth rates between the media for the bacteria caused concern. Since each parameter requires pre-incubation for a set amount of time before running, the bacteria in LB would be nearing stationary phase while the bacteria in either S-medium would be entering exponential growth or in late lag phase. Thus, all bacteriophage parameter estimates are going to be rerun. For the lysogen probability assays and the induction rate assays, the equations of how each parameter is calculated may also be changed to get a better comparable estimate for each media.

However, marine phages are noted to have a decreased burst size and increased latent period when in an environment that is nutritionally limited or deficient the burst size of the as the bacteria are not in optimal growth conditions (20). Lambda phage has a plastic life history, and its parameters can shift between environmental conditions (1). Cellular stress has been noted to impact the induction rate by causing a higher probability of the viral repressor to be cleaved leading to induction (1). Additionally, dilution rate has been seen to affect burst size, latent

period, and adsorption rate which changed with the dilution rate in a chemostat (3). With increasing dilution rates, burst size increased, and latent period and adsorption rate decreased (3).

For the 1:1 competition assays, it was seen that the MKM could outcompete the two sensitive bacteria (MGM and DGM), but was not able to outcompete to EMG. Based on the growth curve data, MKM was likely able to outcompete MGM and DGM due to having a higher growth rate and having a shorter lag time. However, EMG has a higher growth rate than the MGM and DGM so it could better compete with the MKM. Additionally, EMG is far more tolerant to lambda phage so if MKM used spiteful attack, the EMG population would not be as affected as the MGM or the DGM. Moreover, as the experiments were run in well-mixed media, the lysogenic bacteria would be more evenly distributed among the susceptible population. Thus, after induction of a lysogen, the phage would have access to susceptible bacteria and would be able to kill them better (21).

Two competition assays should also be run—1:1 DKM versus sensitive bacteria assays and assays between MKM and MGM where the ratios are varied between the initial number of lysogens and sensitive bacteria. The 1:1 DKM assay is to see if a slower growing lysogen allows for the sensitive bacteria to compete, especially since it is possible that the sensitive bacteria are only being outcompeted by MKM due to the difference in growth rates. The ratio assays are to see if changes in initial population numbers of each strain affects the outcome of competition. Lambda lysogens seem to do better when they are invading from rare rather than being the larger portion of the population as spiteful attack is perceived to be more beneficial (21).

The conditions under which spiteful behaviors boost the fitness of the population has been previously studied. An additional factor in the success of spiteful attack by phage is the

population structure of the microbial community. Spiteful attack is more favored in populations where the lysogens are equal to or less than the sensitive bacteria as there are enough susceptible hosts for the phage to infect (21). In environments with low availability of sensitive hosts, spiteful attack is expected to be inefficient, and low levels of prophage induction are expected to benefit the lysogen by minimizing the costs of prophage carriage. For example, in the gut, high prophage induction leads to a decrease in bacterial fitness as there are not enough susceptible hosts for the phage and leads to the selection of lysogeny as the life path (2). If high induction occurs, many of the phage progeny produced by the induced lysogenic bacteria will be unable to infect susceptible hosts so the phage will have a fitness of zero.

From this study, there are many directions that can be taken to better understand lysogeny. First, ratio assays can be run using the slower growing lysogen, DKM to see if that impacts spiteful attack and lysogeny. Secondly, all competition assays were run in liquid media with shaking, allowing for a well-mixed environment— determination of the effect of spatial structure in an environment where the lysogens and sensitive cannot mixed should be tested.

Specifically, the spatial dynamics of the environment affect the potential benefits of the spiteful attack. Spiteful attack has been noted as more effective in unstructured environments as the lysogens are evenly distributed among the phage and more susceptibles can be killed (17). This differs from structured environments where the lysogens cannot be evenly dispersed among the susceptibles, leading to a higher local multiplicity of infection around an activated lysogen, which leads the exposed susceptibles to becoming lysogens (17). In the structured environment, the spiteful attack may therefore not work as well as a method to take over and get more resources as it seems to do in an unstructured environment. The higher multiplicity of infection seen in structured spatial environments may indicate a low number of susceptible hosts and thus

lead to lysogeny rather that lysis (1). It would be interesting to further investigate the role of spatial structure in future studies.

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