

EXPLORATION OF GENOME LENGTH, BURST TIME, AND BURST SIZE
OF *Streptomyces griseus* BACTERIOPHAGES

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Since phages use the host resources to replicate themselves after infection, the different sizes of the phage genome should influence the replication rate. We, therefore, hypothesized that the smaller genomes should burst the cell faster than the larger ones. As well, the shorter genomes would have greater burst sizes because they should replicate faster. Here, we obtained 16 phages of various genome length. All phages were isolated on *Streptomyces griseus* and available in our phage bank at the University of North Texas. We performed one-step growth studies for the 16 phages, as well as determined the host doubling time from its growth curve. The results show that *S. griseus* grown in nutrient broth has a doubling time of 5 hours and 22 minutes. This doubling time is used as a guideline for the phage growth studies. Because the filamentous nature of the host caused several difficulties during the experiment, we isolated single cells by sonication and centrifugation. After the cell number was determined by viable cell count, the cells were infected with each type of phage using a multiplicity of infection (MOI) of 0.5. The results show that phages' burst times range between 45 (± 0 , standard error) and 420 (± 30) minutes and burst sizes from 12 (± 0) to 1500 (± 60). The statistical analyses show that there is no correlation between either genome size and burst time ($R = -0.01800$, $P = 0.97894$) or genome size and burst size ($R = -0.32678$, $P = 0.21670$). We further performed the comparative genomics studies to investigate whether the phages with similar burst times and burst sizes show similar genome structures. The studies show that Eddasa and Lorelei have similar burst times of 45 to 60 minutes and share 52 homologs. For burst size, only Tribute and Blueeyedbeauty that have similar burst sizes of 21-30, and they are genetically related because of the 48 shared homologs. Although this study did not find any correlation between genome size and burst time/burst size, it provides a foundation for further studies to determine what regulates these two traits.

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CHAPTER 1

INTRODUCTION

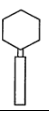






1.1 Bacteriophage

1.1.1 Classification, Morphology, and Structure




Bacteriophages (also known as phages) are prokaryote-specific viruses that were first discovered in 1915 and 1917 by William Twort and Felix d’Herelle respectively (Ackermann, 2007; Brussow, 2005; Edwards et al., 2005). Phages are highly abundant and diverse on our planet. There are 10 families with many different morphologies as shown in Table 1.1. According to the Actinobacteriophage Database (retrieved from www.phagesDB.org), most of the actinobacteriophages belong to the Siphoviridae family, while a small percentage of bacteriophages are classified as Myoviridae, Podoviridae or Tectiviridae.

Table 1.1

Phage classification (adapted from Ackermann 2007).

Shape	Order or family	Nucleic acid	Example	Actinobacteriophage example
	Caudovirales Myoviridae	dsDNA (L), no envelope Tail contractile	T4	Adlitam
	Siphoviridae	Tail long, noncontractile	1	Izzy
	Podoviridae	Tail short	T7	Immanuel3
	Microviridae	ssDNA (C), 27 nm, 12 knoblike capsomers	f X174	
	Corticoviridae	dsDNA (C), complex capsid, lipids, 63 nm	PM2	
	Tectiviridae	dsDNA (L), inner lipid vesicle, pseudo-tail, 60 nm	PRD1	WheeHeim
	Leviviridae	ssRNA (L), 23 nm, like poliovirus	MS2	

(table continues)

Shape	Order or family	Nucleic acid	Example	Actinobacteriophage example(s)
	Cystoviridae	dsRNA (L), segmented, lipidic envelope, 70–80 nm	f 6	
	Inoviridae	ssDNA (C), filaments or rods, 85–1950 x 7 nm	fd	
	Plasmavirida	dsDNA (C), lipidic envelope, no capsid, 80 nm	MVL2	

C; circular, L; linear

Phage T4 is the most well-studied and commonly used phage model for the Caudovirales. According to Yap and Rossmann (2014), phage structures consist of protein molecules including the head capsid proteins, that contain the nucleic acid, neck and collar proteins, body proteins, base plate proteins, and tail fibers proteins as shown in Figure 1.1.

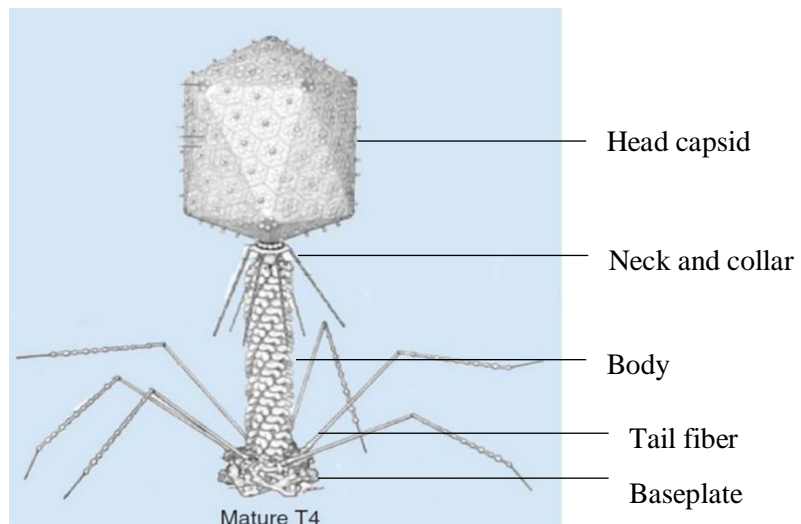


Figure 1.1. Structure of mature phage T4 (Yap and Rossmann 2014)

1.1.2 Phage Life Cycle

Study of phage growth is one of the crucial tools used to gain insight into the biological system. Phages have two life cycles, lytic and lysogenic. Phages that enter the lytic cycle are called lytic phage, whereas ones entering the lysogenic cycle are temperate phages (Martha et al., 2011, Brussow, 2005, Nicastro et al., 2016). Most phages are lytic phage, which lyse the bacterial cell wall of all successfully infected cells and release progeny phages into the

environment. This final step leads to cell lysis and death of the host bacteria (Hyman et al., 2009; Brussow, 2005). A smaller percent of phages is temperate and can undergo and maintain the lysogenic state for thousands of generations (Brussow, 2005; Nicastrò et al., 2016). One unique feature of temperate phages is genome insertion. After the phage injects its nucleic acid into the host cell, the phage genome is inserted into the host genome. This is one aspect of importance for studying genetic diversity in phages.

In order to study phage life cycle in detailed, we need to take a closer look at the molecular mechanisms occurring between a phage and its host which is shown below in terms of stages of infection.

1.1.2.1 Stages of Infection for Lytic Phages (Nicastrò et al., 2016; Hyman et al., 2009; Abedon, 2012)

As shown in Figure 1.2, after a lytic phage meets with its host, there are several molecular events occurring inside the cell.

- Phage adsorption: single or multiple phages bind to receptors on the bacterial cell surface, the phages then inject their genetic material into the host cytosol.
- DNA replication: by using the host resources and phage's proteins, DNA is replicated separately from the host genome.
- Protein synthesis: phages proteins are transcribed and translated into phage-specific proteins such as major capsid protein, tail fiber, endolysin, peptidoglycan, RecA DNA recombinase, etc. Genes expressed at the initial stage of infections are called early genes. They usually encode proteins that regulate protein expression of other molecules such as assembly-related genes and lysis genes. Some of the early genes also regulate the host molecular mechanisms to take over it, for example, *cI*, *cro*,

N. On the other hand, genes encoding proteins that are regulated by early genes are called late genes (Mioduser et al., 2017).

- Phage assembly: phage progenies are produced by an assembly of capsid proteins and nucleic acid. The virions, as well as holin and endolysin proteins, are accumulated in the host cytosol and membrane respectively.
- Cell lysis: when holin is making pores in the inner membrane, it causes proton motive force which releases and activates endolysin. Afterward, activated endolysin migrates from the cytosol to catalyze peptidoglycan. Once the cell wall is lysed, progeny particles are liberated into the environment, ready to infect new host cells (Xu et al., 2004; Hyman et al., 2009; Young, 2014).

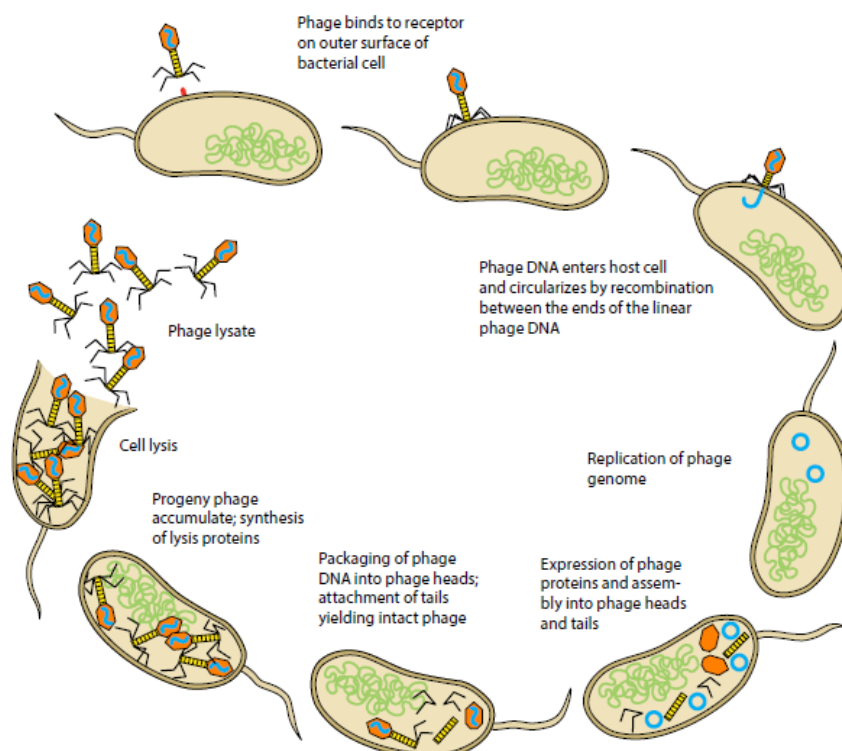


Figure 1.2. Lytic phage cycle (Science Education Alliance Howard Hughes Medical Institute, 2013)

1.1.2.2 Stages of Infection for Lysogenic Phages (Nicastro et al., 2016; Hyman et al., 2009; Abedon, 2012)

After the phage adsorbs to the cell and injects its nucleic acid to the cell cytosol, there are several molecular events occurring during the lysogenic state as shown below.

- DNA recombination: the phage genome recombines to become a part of the host genome. Integrase is required in this process. Once the integration is successful, the phage genome is called prophage (Figure 1.3).
- Protein synthesis: there are some genes expressed during the lysogenic cycle such as immunity repressor. The gene function is to prevent the phage from entering the lytic cycle so that they can maintain the lysogenic state (Defais et al., 1983; Lundqvist and Bertani, 1984).
- Host cell division: when the bacterial cell undergoes protein synthesis and binary fission, the daughter cells inherit the phage genome. The cells having prophage are called lysogeny.

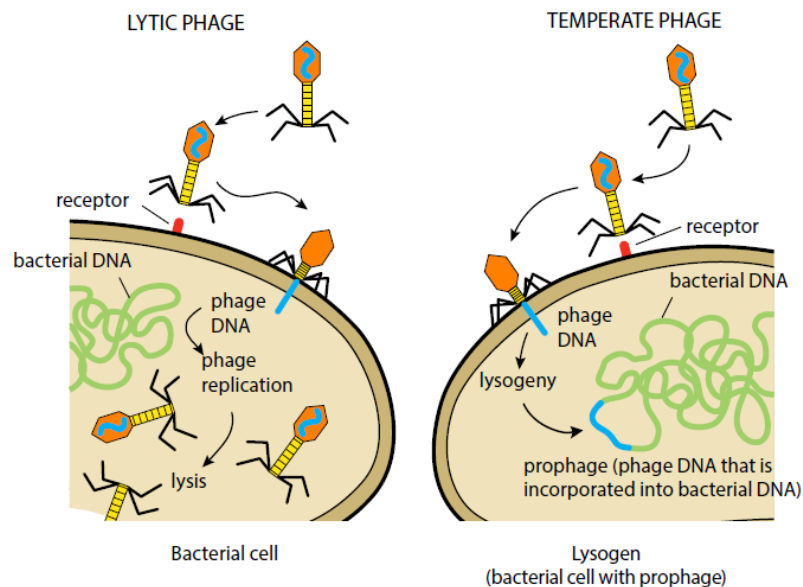


Figure 1.3 Stages of infection for lytic and temperate phage: The lytic phage replicates itself inside the host followed by assembly and lysis. The temperate phage does not replicate the DNA but inserts the genome and becomes a prophage within the bacterial DNA (Science Education Alliance Howard Hughes Medical Institute, 2013).

1.1.3 Phage Growth Terminology

When phage growth is taken into consideration, eclipse period, latent period (so-called burst or lysis time), rise period, plateau period, and burst size are involved. Eclipse period begins from infection to the time right before progeny particles are assembled. During this time, the graph will show a stable level of phage particles (plaque-forming unit: pfu). At the end of the eclipse period, the phage progenies begin to accumulate within the cell. If we combine the eclipse period to the period before burst (or post-eclipse), the extended period is now called “latent period” (Figure 1.4). In this study, the latent period is equivalent to burst time or lysis time which means the time right before lysis begins.

Since all infected cells do not burst at the same time, the rise period is observed (Figure 1.4B). During this time, a significant increase in the number of phage particle can be detected. The end of the rise period is when it reaches the plateau period which is involved in burst size determination.

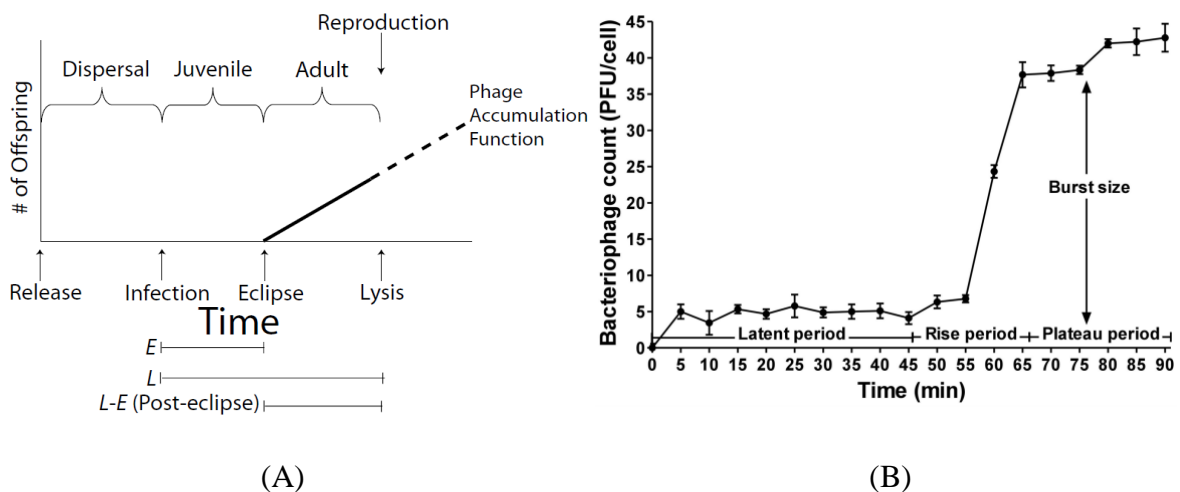


Figure 1.4. Phage growth terminology: (A) Different stages before and after infection; eclipse period starts after infection to phage assembly (Heineman and Bull, 2007). (B) Latent period, rise period, plateau period, and burst size (Gwak et al., 2018)

Burst size is the number of progeny particles that are released from one bacterium. The total number of liberated phage particles shown at the plateau period is used to determine burst size.

Thus, burst size = $\frac{\text{Number of liberated phage particles}}{\text{Number of infected bacterial cells}}$ Generally, burst size is reported without a unit (Hyman et al., 2009).

1.1.4 One-Step Growth

One-step growth experiment was first proposed by Ellis and Delbruck in 1939. It is an important method used to study phage growth and its parameters are recently mentioned in

1.1.3. Basically, this method is designed to avoid multiple cycles of phage growth.

The protocol described by Hyman et al. (2009) is comprised of adsorption and synchronization, post-adsorption dilution, eclipse and latent period determination, and stand-alone burst size.

The first step of this experiment is to infect the host cells with phage particles (or phage adsorption). An appropriate phage-to-host ratio, or multiplicity of infection (MOI), is employed. When using MOI of 0.1 (1 phage to 10 bacterial cells), 95% of infected cells will be infected by only one phage particle. This is important to determine unadsorbed cell number, and also to control the number of phage particles that infect the cell. After phages are added to host bacteria, the suspension will be incubated for a short period of time to allow the phages to adsorb and inject their genetic material into the cell.

After a quick incubation (10-15 minutes), the suspension will be diluted to reduce the chance of multiple adsorptions. This is very crucial because (1) it is a synchronization meaning that phages failing to adsorb during the short incubation will be prevented from further infections and (2) it prevents the system from multiple adsorptions as the progeny phages fail to meet new hosts. In addition to the dilution, free phage removal will be performed, if needed.

The diluted suspension is then incubated for a long period of time. Samples will be taken at each time interval. A released phage titer will be determined subsequently to observe the phage growth over time. In this step, if a sudden rise of the phage titer is detected, it will be called either “end of constant period” or “minimum latent period”. Individual cell lysis time can be varied, this is a reason why there is a “rise period” shown in the one-step growth curve (Figure 1.5).

For burst size determination, the ratio of phage titer at the end of the rise period to the number of infected cells will be computed as described previously.

Nevertheless, we have to be aware that one-step growth is best employed to study phages and their single cell hosts and this process is complicated when studying a host such as *Streptomyces* which grows through production of hyphae or mycelia.

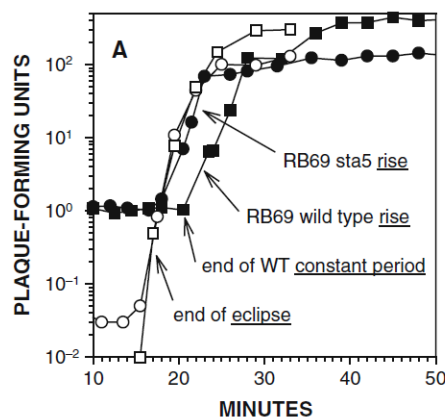


Figure 1.5. Eclipse and latent period for RB69 (WT) and RB69 sta5 (mutant) phages (Hyman et al., 2009)

1.1.5 Cell Lysis Mechanism

Many previous studies have shown that phage lysis time is triggered by the holin-endolysin system. The two proteins are encoded by the phage genome. The endolysin is a small muralytic enzyme with an approximate molecular mass of 18 kDa (for gram-negative bacteria phages) (Xu et al., 2005) and 25-40 kDa (for gram-positive bacteria phages) (Fischetti, 2010). It is produced in an inactive form such that the N-terminus, so-called signal arrest and release (SAR) domain, is embedded in the inner membrane (Figure 1.6).

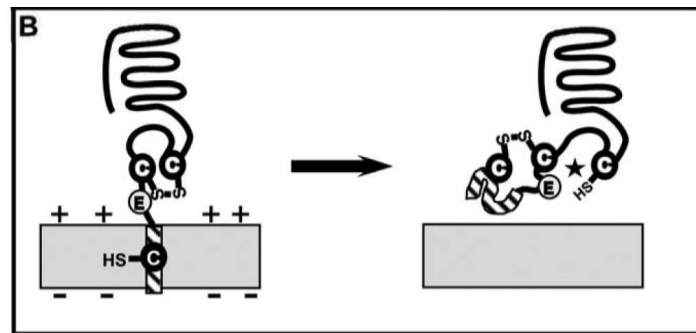


Figure 1.6. Phage P1 endolysin activation in the periplasm of *E. coli* (Xu et al., 2005)

At the genetically scheduled time, holin, a membrane protein, makes holes in the inner membrane. The proton motive force from this process activates the endolysin by releasing it from the membrane. Once the protein is free, it migrates to and catalyzes the peptidoglycan layer resulting in cell lysis (Young, 2014).

1.2 Streptomyces

In this study, we are interested in *Streptomyces* phages because the University of North Texas is one of the Howard Hughes Medical Institute’s Science Education Alliance-Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) institutions and through this program has developed a large *Streptomyces* phage bank. From this project, more than 400 phages available for *Streptomyces* phages have been isolated (retrieved from <http://phagesdb.org/institutions/UNTD>). Additionally, we found no reports regarding a correlation between *Streptomyces* phages’ genome length and their burst time and burst size (Table 1.2).

Table 1.2

Gathered data from several hosts regarding burst time and burst size

Phage	Host	Phage genome length (kb)	Burst time (min)	Burst size	References
HWPB-1	<i>P. aeruginosa</i>	N/A	100	392.8 ± 220.1	Lindberg, McKean, and Wang (2014)
HWPB-2			50	260.0 ± 101.9	
HWPB-3			93±7	36.8 ± 15.1	
HWNPB-1			70±6	589.4 ± 12.6	
HWNPB-2			47±3	246.1 ± 55.3	
HWNPB-3			47±3	116.5 ± 42.5	
P1	<i>L. plantarum</i> IMAU10120	73.8	90	132.88 ± 2.37	Chen, et al. (2016)
φX174	<i>E.coli</i>	5.4	12.2	N/A	Baker, et al. (2016)
T7	<i>E. coli</i> IJ1133	40	13.3	266 to 327	Heineman and Bull (2007)
PHq0	<i>Pseudoalteromonas</i> BQ0	33.4	15	363	Wang et al. (2016)
Kampy	<i>M. smegmatis</i>	51.4	95-100	N/A	Halleran, et al. (2015)
ΦStp1	<i>Salmonella</i>	N/A	30	37	Sritha and Bhat (2018)
514-3	<i>S. griseus</i>	N/A	120	75-100	Gilmour, et al. (1959)
VP11	<i>S. coelicolor</i>		40	40	Dowding (1973)

1.2.1 Taxonomy and Cell Structure

The Genus *Streptomyces* are gram-positive bacteria classified in the family *Streptomycetaceae*, phylum Actinobacteria (Stackebrandt et al., 1997). Streptomycetes are unusual bacteria because they are not a single cell prokaryote but produce 0.5-2 μm mycelium (Figure 1.7A) and have moldlike growth habits. There are two types of mycelia: substrate and aerial. Substrate mycelia grow from a germinating spore on the agar surface and produce pigments, while aerial mycelia grow into the air and develop hyphae that can produce spores (Wink et al., 2017).

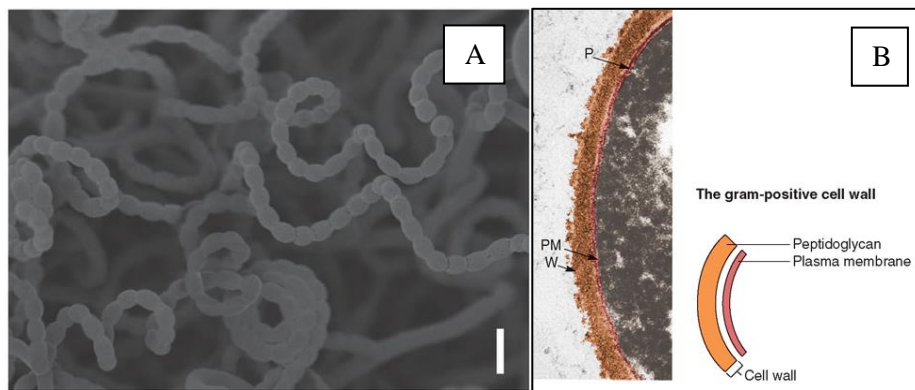


Figure 1.7. Streptomycete cell structure: (A) Cryoscanning electron micrograph of *S. coelicolor* M145 aerial hyphae (Zhang et al., 2016). (B) The gram-positive cell wall structure (Brooks et al., 2012).

1.2.2 Streptomycete Life Cycle

According to Figure 1.8, when a mature spore is in the appropriate conditions, such as optimum temperature, humidity, and nutrients, it will germinate and develop substrate mycelium by branch extension. It has been reported that when spores are inoculated, RNA and protein syntheses occur within 5 minutes (Mikulík et al., 1984). After 30 hours of inoculation, the aerial mycelia have developed and produced several bioactive agents known as secondary metabolites. When the morphological differentiation has begun, the aerial mycelia grow into

the air and after 2 days, they stop growing branches. At this point, prespores are produced from the septation of the mycelia. The prespores will then differentiate into mature spores.

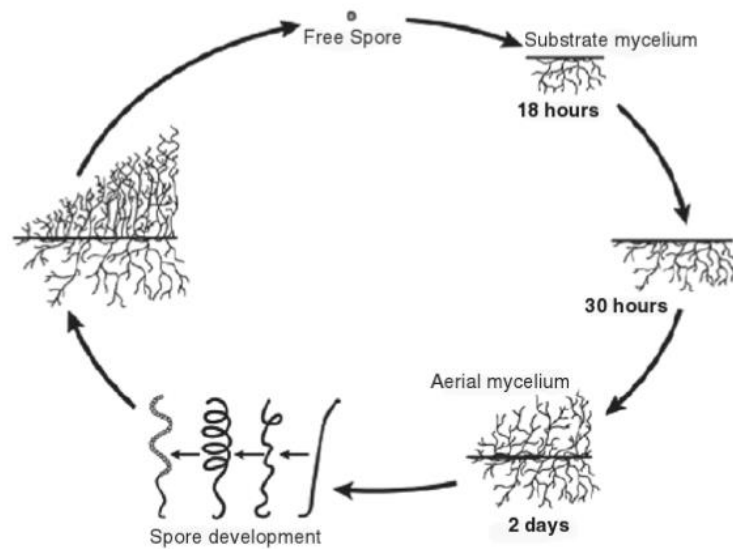


Figure 1.8. *Streptomyces coelicolor* life cycle (Angert, 2005)

1.3 Importance of this Study

As described earlier, we found no prior reports regarding a relationship between *Streptomyces* phages' genome length and their burst time or burst size (Table 1.2). In this study, we would like to get insights into *Streptomyces* phage biology by providing a one-step growth protocol for the filamentous hosts.

1.4 Hypotheses

During the lytic cycle, genome length should affect the replication and protein synthesis rate because the longer genomes would take longer to replicate as well as use more resources from the host cells. As the longer genomes would take longer, their burst size would be smaller. We, therefore, developed hypotheses as shown below.

1.4.1 Shorter phage genomes will burst the host faster than larger phage genomes, and vice versa.

1.4.2 Shorter phage genomes will have greater burst size than larger phage genomes, and vice versa.

1.5 Research Objectives

1.5.1 To determine the infected cell incubation period by using *S. griseus* doubling time.

1.5.2 To determine burst time and burst size for 16 phages isolated on *Streptomyces griseus* (ATCC 10137).

1.5.3 To evaluate the relationship between burst time and phage genome length.

1.5.4 To evaluate the relationship between burst size and phage genome length.

1.5.5 To perform comparative genomics within the same phage group of burst time and burst size.

1.6 Scope of Research

1.6.1 Determine *S. griseus* growth curve and doubling time.

1.6.2 Make high-titer lysate of 16 phages isolated on *S. griseus* ATCC 10137 (phage list shown in Table 2.1).

1.6.3 Perform a one-step growth method for lysis time and burst size determination using 16 phages.

1.6.4 Perform the comparative genomics between groups of burst time and burst size.

CHAPTER 2

METHODOLOGY

2.1 Bacterial Culture and Condition

2.1.1 Bacterial Strain and Growth

Streptomyces griseus (ATCC 10137) was used as the bacterial host for the entire study. The bacterial culture was grown in nutrient broth and agar (Difco™ Nutrient Broth and Difco™ Agar, Granulated; Becton, Dickinson and Company) supplemented with media supplement (3 mM MgCl₂, 2 mM Ca(NO₃)₂, and 24 mM glucose). The liquid culture was incubated for 24 hours in the 30°C New Brunswick Scientific G-25 incubator shaker with a speed of 210 rpm, while the bacteria on agar was incubated for 24-36 hours in the 30°C Thermo Scientific™ HeraTherm incubator.

Doubling time and growth curve of this strain were determined by using dry weight method. 15 mL of log-phase culture was inoculated into 1.5 L of nutrient broth. 50- mL samples were taken every 4 hours up to 72 hours. The samples were then processed immediately after each collection, otherwise, it was held at 4°C. Each sample was filtered through a pre-weighed 0.45 µm filter membrane which was dried at 35°C for 120 hours. The membrane was weighed afterward to compute bacterial dry weight at each time point. The data was gathered to create a growth curve and determine bacterial doubling time.

2.1.2 Single Cell Generation

S. griseus single cells were used for the one-step growth experiment. They were generated by using sonication and centrifugation. 350 µl cultures were diluted with 6 mL nutrient broth (supplemented with media supplement). The diluted suspension was subsequently sonicated for 3 minutes. Since the sonication generates heat, the suspension was sonicated only 1 minute at a time with 3-4 minutes breaks. The power output used was 4 Watts (RMS). The sonicated cell suspension was then spun at 300 x g for 3 minutes to separate remaining clumps. The supernatant was collected, and cell counting was performed.

2.1.3 Cell Counting

Cell number was determined by microscopy. The sonicated cell suspension was diluted 10 times using phage buffer then dyed with 0.1% methylene blue with a 1:1 ratio. The stained suspension was loaded into both hemocytometer chambers, and the cells were counted under a light microscope with a 400X magnification. See Figure 2.1 for the counting areas.

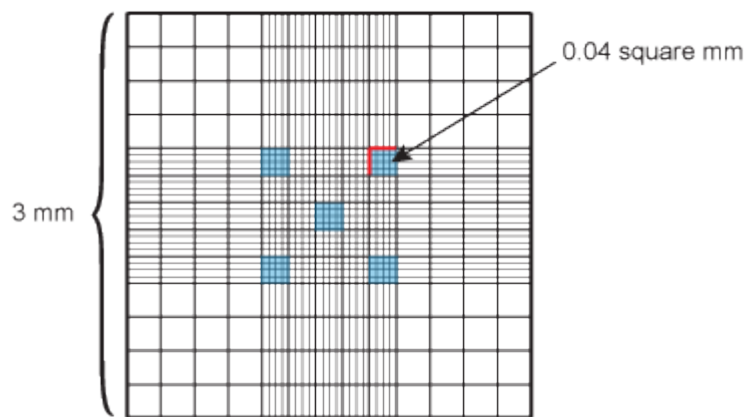


Figure 2.1. Gridlines in hemocytometer chamber. The blue squares are the counting areas. (Retrieved from http://dictybase.org/techniques/media/dicty_growth.html)

After counting the cells in the corresponding areas, a cell number was determined as shown below.

$$\text{Cell number} = \text{Average cell count} \times 5 \times 2 \times 10 \times 10^4 \text{ cell/mL}$$

Note that x5 to obtain the total count from 25 squares, x2 because the cells were stained with 0.1% methylene blue with 1:1 ratio, x10 because the cell suspension was diluted 10 times, and x10⁴ to convert 0.1 cu.mm. to 1 mL.

2.2 Phage Selection and High-Titer Lysate

16 *S. griseus* phages were selected for one-step growth studies (Table 2.1). Before testing, all phage titers were determined by using the plaque assay. High-titer lysates were made if needed. The protocol for lysate harvesting is described in the STREPTOMYCES PHAGE: Laboratory Manual. (Science Education Alliance Howard Hughes Medical Institute, 2013). Lysates containing phage buffer (0.01 M pH 7.5 Tris-HCl, 0.01 M MgSO₄, 4 g of NaCl, 0.01 M CaCl₂, and 980 mL of distilled water) were stored at 4°C.

Table 2.1

Bacteriophages used in this research

Phage name	Genome length (kbp)	Cluster
Raleigh	40.7	BC2
Percastrophe	46.0	BF
ToriToki	46.1	BF
Romero	46.1	BF
Immanuel3	46.1	BF
BryanRecycles	50.1	BD1
Izzy	50.1	BD1
Lorelei	50.6	BD1
Eddasa	50.6	BD1
Wentworth	68.3	BN
Gibson	69.4	BN
Comrade	129.0	BK1
Blueeyedbeauty	130.5	BK1
NootNoot	131.1	BE1
Karimac	131.9	BE2
Tribute	133.5	BE1

2.3 Preliminary Burst Time Determination

In this experiment, a certain number of cells was infected using an unknown MOI. In order to use the same number of cells, we froze equal aliquots of culture (1 mL) in microcentrifuge tubes.

2.3.1 Freezing Cells

Log-phase of *S. griseus* culture, grown in nutrient broth, was frozen in glycerol. It was started from spinning 2 tubes of 47 mL culture at 1,800 x g for 10 minutes. The pellet in each tube was resuspended in 15.7 mL phage buffer after the supernatant was removed. Afterward, the cell suspension in both tubes was resuspended and 18.75 mL of 80% glycerol was added to produce the final concentration of 25-30% glycerol (Shepherd et al., 2010). 1 mL of the cell-glycerol suspension was then transferred to each microcentrifuge tube and frozen at -20°C. The frozen suspension will be expired within a year.

2.3.2 Thawing Frozen Cells

The frozen cell suspension was thawed at 37°C for 1-2 minutes. Thawed suspensions were spun at 10,000 x g for 5 min to remove glycerol. The pellet was resuspended in nutrient broth (with media supplement) for experimentation.

2.3.3 Burst Time Determination

This experiment was performed with 3 replicates for each phage studied.

Adsorption: Thawed *S. griseus* was infected by phage with a titer of 10^8 to 10^9 pfu. The mixture was incubated in a rotary shaking incubator at 30°C for 15 minutes to allow phage adsorption.

Removing unadsorbed phages: Immediately after 15-minute adsorption, the cell-phage mixture was centrifuged for 10 minutes at 1800 x g. Unadsorbed phages in the supernatant were discarded. The pellet was washed (resuspended and centrifuged) with 5 mL of phage buffer for 3 times.

Phage growth and sample collection: Washed pellets were then transferred to 25 mL of nutrient broth (with media supplement) and incubated at 30°C for a long period of time. 1-mL samples were collected every 30 minutes from 0 hours (T0) to 6 hours (T6).

Sample processing: The collected samples were filtered through 0.2 μm syringe filters immediately after collection. The phage titer was determined by the spot test.

2.4 One-Step Growth Studies (Adapted from Gilmour et al., 1959)

As mentioned above, this experiment and the preliminary burst time study are similar to each other. In this experiment, single cells were generated; they were infected with an MOI of 0.5. Furthermore, we controlled the temperature during spinning after adsorption to halt the intracellular activities by changing two steps. (1) The cell-phage suspension was spun down for 5 minutes at 3,500 x g, 4°C. (2) The pellet was washed 3 times with refrigerated phage buffer. Afterward, the pellet was resuspended in 1 mL nutrient broth and diluted to prevent multiple infections as shown below.

One-step growth: 1 mL of the washed pellets were transferred to a tube containing 9 mL of nutrient broth. The tube is labelled as 10^0 . It is then diluted down to another tube labelled as 10^{-1} . Before incubation, a 0.3-0.5-mL aliquot was removed from each tube for phage titer determination at T=0 and infected cell number assay. The infected cell suspensions were subsequently incubated in the shaker at 30°C for several hours, depending on the preliminary burst time results. The samples were collected every 15 minutes (Table 2.2).

Table 2.2

Sample collection at each time point: 4 groups of phages divided based on the approximate burst time resulted from the preliminary study. The first group's samples were collected every 15 minutes from T=0, while other groups have a gap of about 1-2 hours. All samples include the sample for the infected cell assay, and the volume for all samples is 300-500 µl.

Group	Phages	Approximate burst time (minutes)	Duration of sample collection (minutes)	Infected cell*	Sample volume (µl)
1	Izzy, BryanRecycles, Lorelei, and Eddasa	60	0-300	✓	300-500
2	Romero, Tribute, and NootNoot	90-120	0, 60-360	✓	
3	Raleigh, Wentworth, Gibson, Immanuel3, Blueeyedbeauty, Karimac, and Comrade	150-210	0, 60-420	✓	
4	Percastrophe and ToriToki	300-330	0, 150-540	✓	

*Samples for infected cell assay were non-filtered and collected at T=0.

Sample processing and phage titer determination: All the samples, except ones for infected cell assay, were filtered through a 0.2 µm syringe filter immediately after collection. At least 2 plates of a standard plaque assay were done to determine phage titer for infected cell assay, while a spot test (Science Education Alliance Howard Hughes Medical Institute, 2013) was used to determine phage titer at each time point. Samples for infected cell assay were plated immediately after collection. Computation for phage titer is shown below.

$$\text{Phage titer (pfu)} = \frac{\text{Average number of plaque} \times \text{dilution factor} \times 10^4 \mu\text{l}}{\text{volume of sample } \mu\text{l}}$$

Note that 5 and 50 µl are the volumes of sample used in spot test and plaque assay, respectively, and 10⁴ µl is the total volume in the culture tube (10 mL).

Burst size determination:

As mentioned in 1.1.3, $Burst\ size = \frac{Number\ of\ liberated\ phage\ particles}{Number\ of\ infected\ cells}$

In this experiment,

$Number\ of\ liberated\ phage\ particle = phage\ titer\ after\ burst - free\ phage\ at\ T = 0$

Similarly,

$Number\ of\ infected\ cells = phage\ titer\ from\ infected\ cell\ plates - free\ phage\ at\ T = 0$

2.5 Statistical Analyses

Coefficient correlation (R-value) and ANOVA (P-value) were used in the pairwise analysis between genome size-burst size and genome size-burst time. Additionally, GC content-burst size, GC content-burst time, genome size-GC content, and burst time-burst size were tested. Microsoft Excel is the software we used to compute the two values.

2.6 Bioinformatic Studies

16 phages were grouped based on their burst times and burst sizes, with the range of 20 minutes, and 10 in relation to burst size. The phage genome maps are generated by Phamerator. The pairwise analysis of the two genomes that show similar burst time or burst size was performed. Purple shade on the maps shows the most nucleotide similarity while the white indicates no similarity. Furthermore, all the homologs shared between the two genomes were numbered.

CHAPTER 3

RESULTS

3.1 *Streptomyces griseus* Growth and Doubling Time

S. griseus (ATCC 10137) is the host of all phages used in this study. The results show that the strain has an approximate doubling time of 5 hours and 22 minutes for the media and culture conditions used in this study.

According to Figure 3.1, the lag phase occurs in the first 4 hours of incubation, followed by the log phase. The bacteria tend to be in the stationary phase after 12-20 hours of inoculation, although there is an increase in the dry weight during this phase. This could be because there are some cells continue growing while a slightly smaller number of cells starts to die.

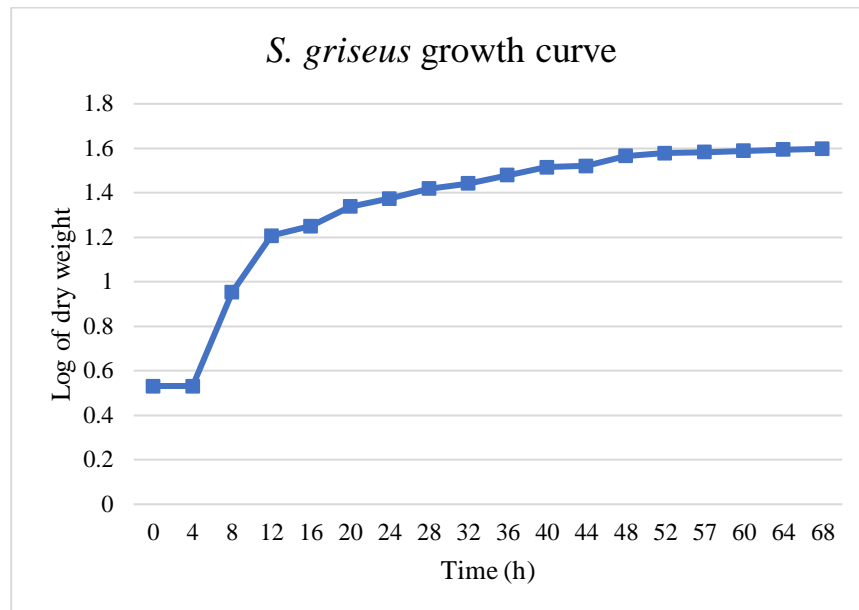


Figure 3.1. *S. griseus* growth curve determined by using dry weight

Importantly, the results show a doubling time of 5.36 hours. Dry weights at T=0 and 12 were computed by using a growth equation described as:

$$X = 2^n X_0$$

In the above equation, X is the dry weight (mg) at T=12, n is the number of generations, and X_0 is the dry weight (mg) at T=0. X and X_0 are 16.1 and 3.4 mg, respectively. Therefore, the number of generations over 12 hours is 2.24. This results in a doubling time of 12 hours/2.24 generations which is 5.36 hours/generation or 5 hours and 22 minutes as mentioned above.

Due to the host's approximate doubling time, the phages used in this study were incubated for at least 5 hours to determine burst time and burst size.

3.2 Burst Times and Burst Size for the 16 Phages

There were 16 phages used in this study. All of them were isolated on *S. griseus* by several scientists and students at the University of North Texas from 2012 to 2017. After DNA sequencing, they were grouped into 7 different clusters and subclusters including BC2, BF, BD1, BN, BK1, BE1, and BE2. Their genome sizes varied from 40.7 to 133.5 kbp. (Table 3.1).

According to Table 3.1, burst times and burst sizes for the 16 phages range from 45 (± 0 , standard error) to 420 (± 30) minutes and 12 (± 0) to 1500 (± 60), respectively. Interestingly, the two largest burst sizes belong to the smallest phages (Raleigh and Percastrophe), and they have burst times of 185 (± 9) and 420 (± 30) minutes.

Table 3.1

Burst times and burst sizes for 16 phages sorted by genome size.

No.	Phage name	Genome size (kbp)	Cluster	GC content (%)	Burst time (minutes)	Burst size
1	Raleigh	40.7	BC2	71.8	185±9	1000±577
2	Percastrophe	46.0	BF	59.7	420±30	1500±60
3	Immanuel3	46.1	BF	59.6	145±8	240±49
4	Romero	46.1	BF	59.7	120±0	12±0
5	ToriToki	46.1	BF	59.7	345±0	190±62
6	BryanRecycles	50.1	BD1	65.9	210±0	190±97
7	Izzy	50.1	BD1	65.9	80±17	22±5
8	Eddasa	50.6	BD1	65.9	45±0	32±0
9	Lorelei	50.6	BD1	65.8	80±17	20±10
10	Wentworth	68.3	BN	64.1	275±31	30±7
11	Gibson	69.4	BN	64.4	120±0	55±0
12	Comrade	129.0	BK1	47.1	150±0	32±0
13	Blueeyedbeauty	130.5	BK1	47.9	285±15	21±7
14	NootNoot	131.1	BE1	50.2	245±9	270±173
15	Karimac	131.9	BE2	49.4	180±0	20±3
16	Tribute	133.5	BE1	50	105±0	26±0

However, the data for the entire population shows that burst time and burst size do not correlate with genome size with the scores of -0.01800 and -0.32678, respectively. When GC content was compared to burst time and to burst size, the results also showed no correlation with scores of -0.15391 and 0.22644, respectively. Nonetheless, burst time does show a weak correlation with burst size with a score of 0.56341 (see Table 3.2).

Furthermore, when the correlations within a group (groups of 40, 50, and 130 kbp genome size) were tested, the results showed that the genome size of 50 kbp is correlated to burst time and burst size with the scores of -0.70353 and -0.55760, respectively. Also, burst time and burst size do show correlation in the groups of 40 and 50 kbp as shown in Table 3.2. The group of 60 kbp was not statistically tested due to population insufficiency.

Table 3.2

Statistical analysis: *only one pair shows a correlation (R) with $P < 0.05$. For the entire population, genome size-burst time and genome size-burst size do not show any correlation. In the group of 50 kbp, there is a correlation between genome size-burst time and burst time-burst size. Generally, correlation must be close to 1 or -1. The group of 60 kbp is not shown here due to population insufficiency.

Pairwise analysis	R-value	P-value
Entire population		
Genome size – Burst time	-0.01800	0.97894
Genome size – Burst size	-0.32678	0.21670
GC content- Burst time	-0.15391	0.56929
GC content – Burst size	0.22644	0.39904
Burst time – Burst size*	0.56341	0.02305
Genome size of 40 kbp		
Genome size – Burst time	0.23256	0.70658
Genome size – Burst size	-0.37891	0.52935
GC content- Burst time	-0.24146	0.69558
GC content – Burst size	0.36413	0.54683
Burst time – Burst size	0.60158	0.28315
Genome size of 50 kbp		
Genome size – Burst time	-0.70353	0.29647
Genome size – Burst size	-0.55760	0.44340
GC content- Burst time	0.24371	0.75630
GC content – Burst size	0.37022	0.62980
Burst time – Burst size	0.94542	0.05459
Genome size of 130 kbp		
Genome size – Burst time	-0.38658	0.52034
Genome size – Burst size	-0.05231	0.93343
GC content- Burst time	-0.12791	0.83758
GC content – Burst size	0.50875	0.38138
Burst time – Burst size	0.37929	0.52892

The majority of phages (44%) has burst times between 100 and 199 minutes. There is only one phage (Percastrophe) that has a very long burst time of 420 (± 30) minutes. For burst size, the majority of phages (62%) has a burst size of less than 100 (Figure 3.2).

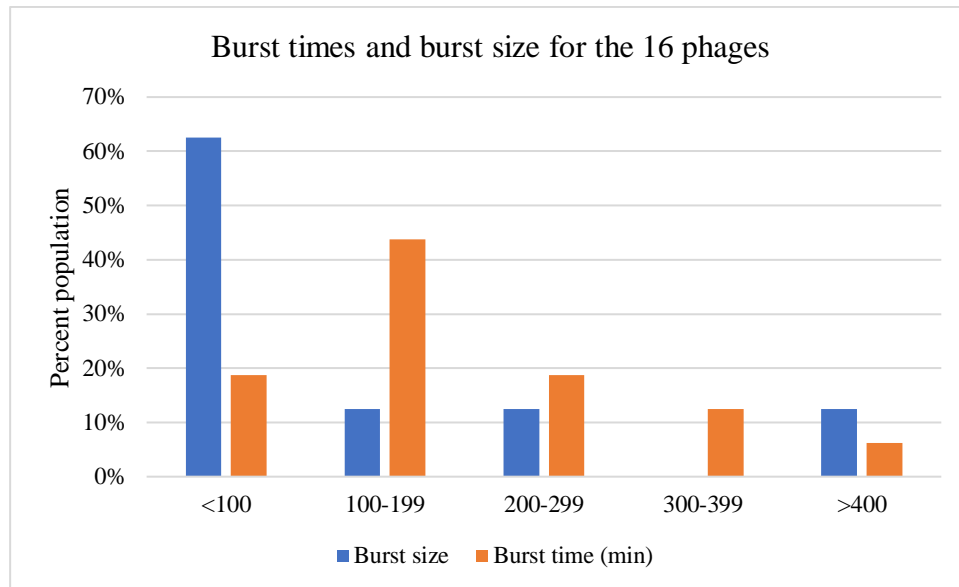


Figure 3.2 Burst times and burst sizes for the 16 phages: 44% of burst times are between 100 and 199 minutes, while 62% of burst sizes are less than 100.

Raleigh, Percastrophe, Immanuel3, Romero, and ToriToki all belong to a group of phages with a small genome size of approximately 40-46 kbp. Their burst times are at least 120 minutes. The longest burst time belongs to Percastrophe which also has the largest burst size, as mentioned before. However, Romero showed the smallest burst size of 12 (± 0). Raleigh had a burst time of 185 (± 9) minutes and the second largest burst size of 1000 (± 577).

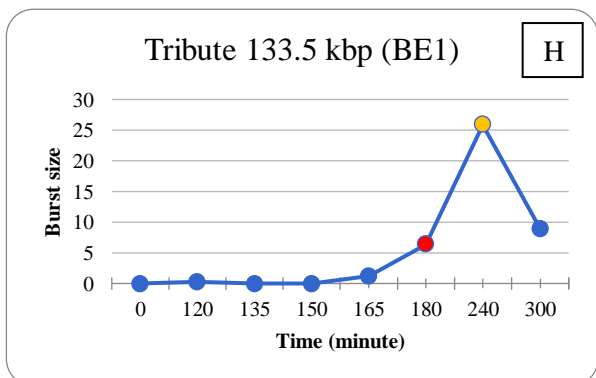
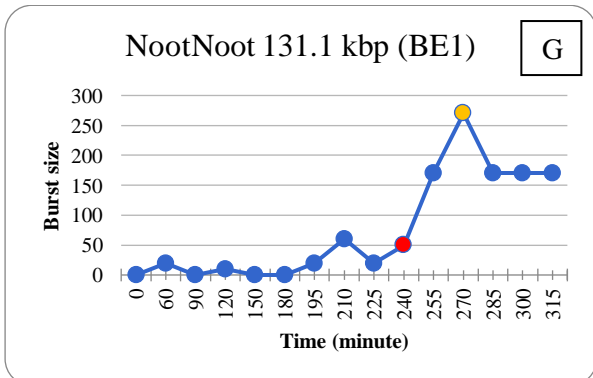
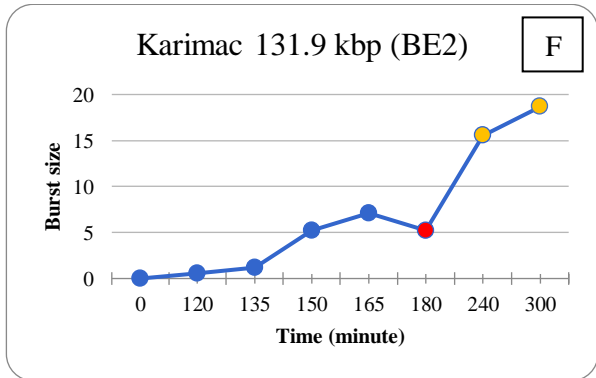
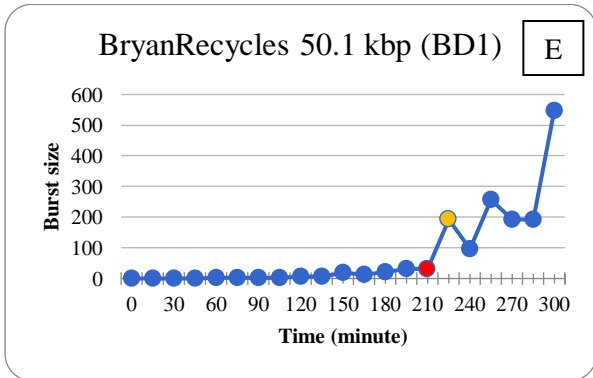
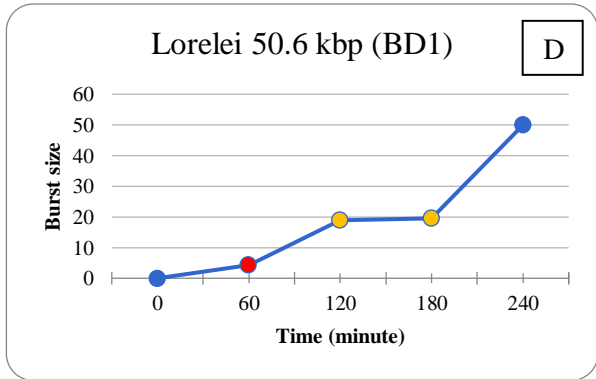
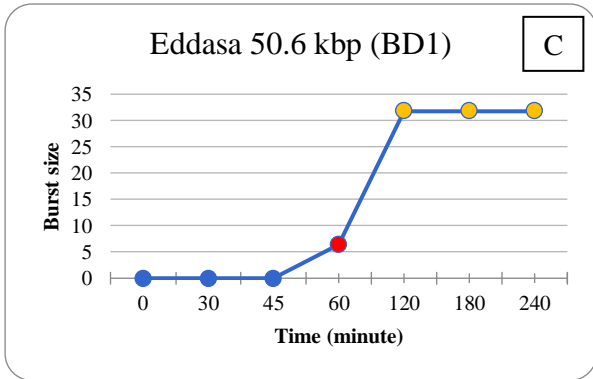
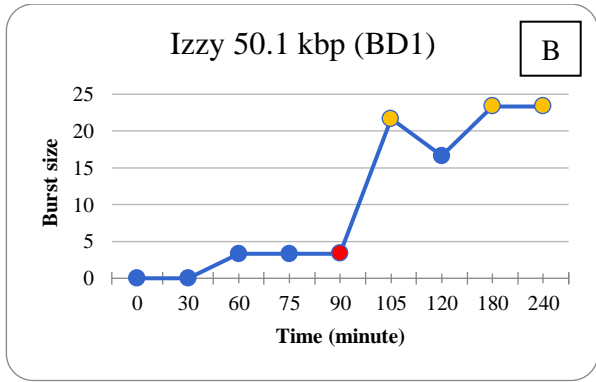
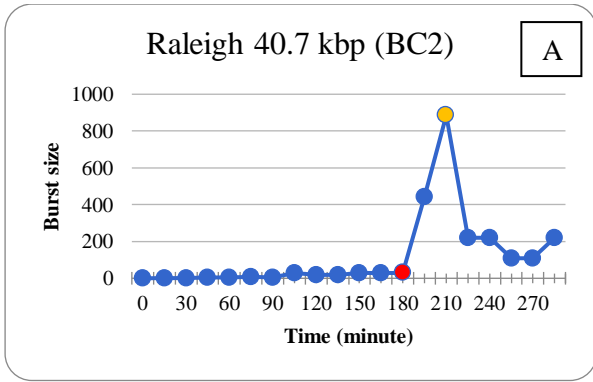
The second group (genome size of 50 kbp) of phages consisted of BryanRecycles, Izzy, Eddasa, and Lorelei. All of them are clustered in BD1. The phages burst times range between 45 and 210 minutes, while burst size is between 20 and 190. Again, the largest burst size is observed from BryanRecycles which has the longest burst time of 210 (± 0) minutes. However, the smallest burst size (20 ± 10) for Lorelei does not show the shortest burst time.

The third group consisted of Wentworth and Gibson, both members of the BN cluster. Even though the two phages are very similar to each other in terms of genome size and GC

content, they show different burst times and burst sizes. Wentworth has a longer burst time of 275 (± 31) minutes but has a smaller burst size of about 30 (± 7).

The last group consists of phage belonging to the BK1, BE1, and BE2 subclusters. This group contains the largest genome size (129-133.5 kbp) and consists of Comrade, Blueeyedbeauty, NootNoot, Karimac, and Tribute. Interestingly, Blueeyedbeauty has the longest burst time of 285 (± 15) minutes but its burst size of 21 (± 7) is not as large. Nevertheless, Tribute has a shortest burst time of 105 (± 0) minutes and it also shows almost the same burst size (26 ± 0) as Blueeyedbeauty's.

During the one-step growth experiment, the number of released progenies were observed over time. The data was used to compute burst size as mentioned in 2.4. The burst sizes measured over time were plotted in the graphs shown in Figure 3.3A to P. All the graphs have a baseline of zero resulting from no burst during the latent period. Once the graphs start the rise period, red dots were labeled as burst time or the very beginning of the rise. However, the yellow dots were labeled as burst size as they are the greatest burst size, or "the peak", observed after the lysis. Most of the graphs show the decreases in burst size after the peak. This reflects the unwanted second adsorption occurring in the biological system.



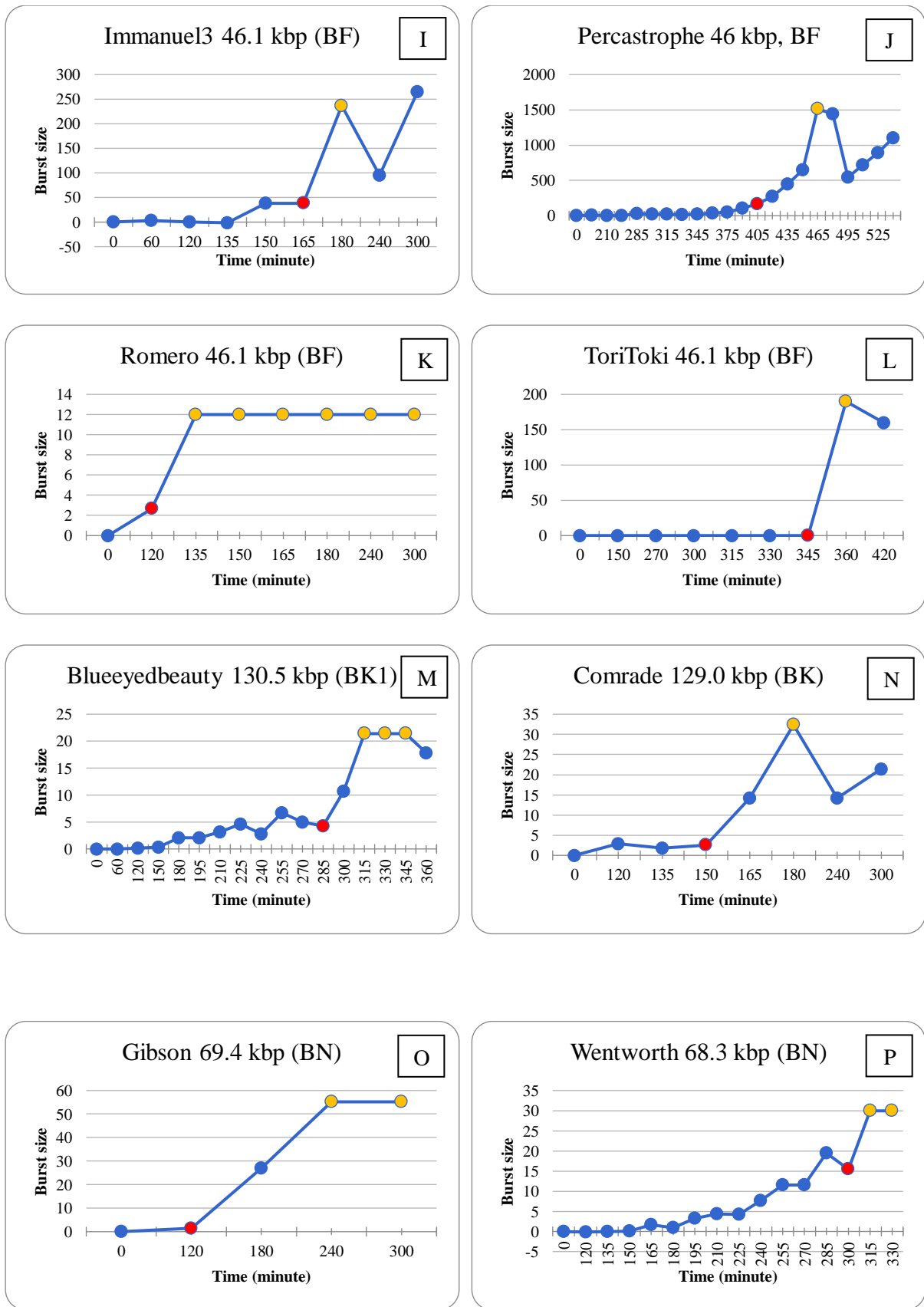


Figure 3.3 (A to P) 16 phages growth curves. Red and yellow dots represent burst time and burst size, respectively.

3.3 Bioinformatics: The Comparative Genomics

Computational studies have been done to further investigate what could be regulating burst time and burst size since genome size and GC content were not correlated. Genetic properties of the bacteriophage were the next part of the project that needed to be investigated.

We grouped the 16 phages into categories based on their burst times and burst sizes. With the burst time range of 20 minutes, the phages can be divided to 4 groups; with a burst size range of 10, the phages can be divided into 3 groups as shown in Figure 3.4 and 3.5. Any grouping in the diagrams below had one member could not be compared genetically.

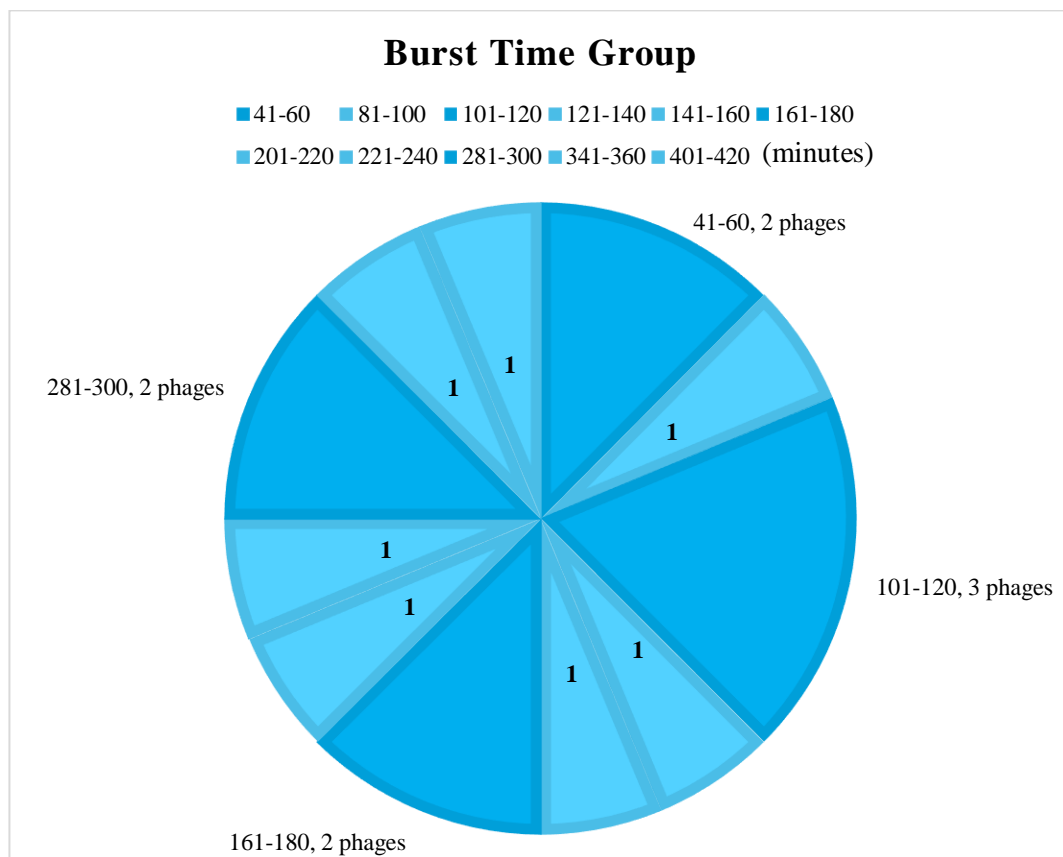


Figure 3.4 Phage groups based on their burst times. There are 2, 3, 2, and 2 phages in the groups of 41-60 minutes, 101-120 minutes, 161-180 minutes, and 281-300 minutes, respectively. 1 indicates that there is only one member; cannot form a group.

Within the same burst time group, we compared the genome structure of the phages in the same group. The first group (41-60 minutes) consists of Eddasa and Lorelei. The second group (101-120 minutes) consists of Tribute, Romero, and Gibson. The third group of 121-140 minutes of burst time includes Karimac and Immanuel3. Lastly, Blueeyedbeauty and Wentworth are grouped together with burst times between 281-300 minutes.

Their genome maps shown in Figure 3.6 to 3.10 suggest that only the first group shows similarities. There are 52 shared homologs out of 76 and 75 genes for Eddasa and Lorelei, respectively (Figure 3.6). The other groups have no nucleotide similarity (Figure 3.7 to 3.10)

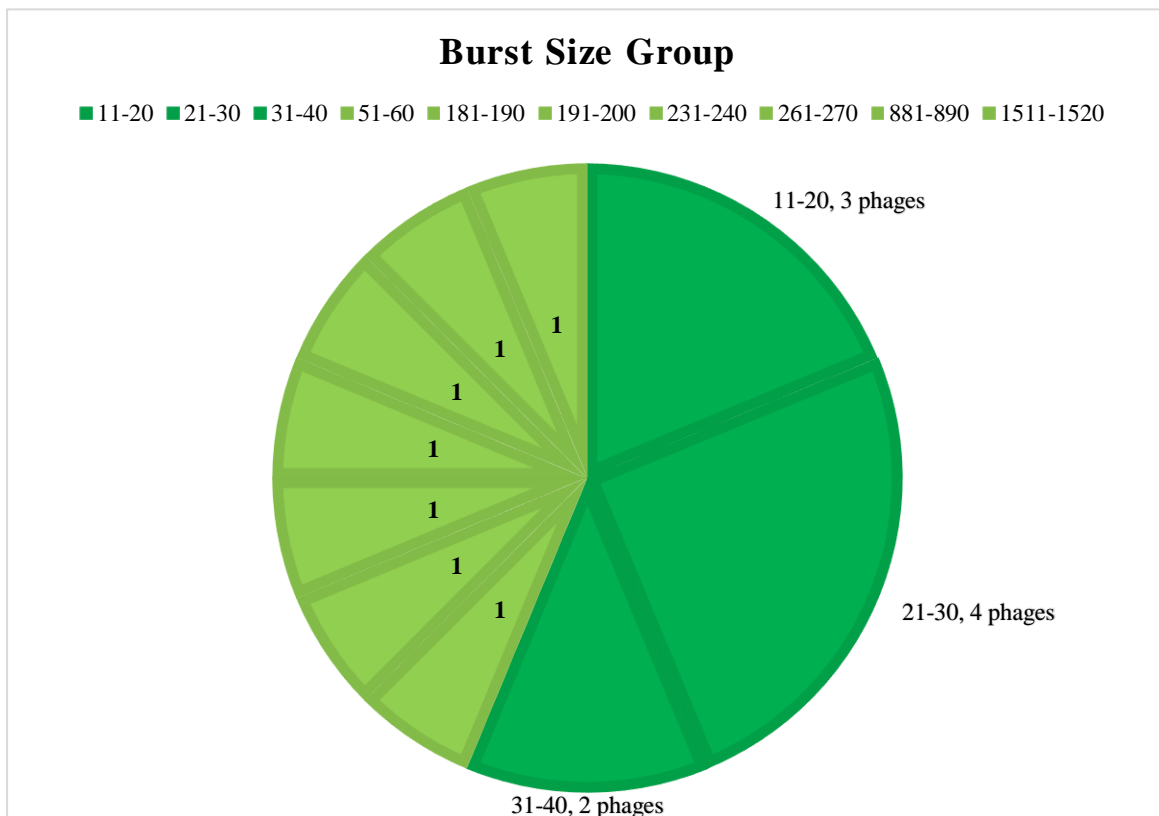


Figure 3.5 Phage groups based on their burst sizes. There are 3, 4, and 2 phages in the burst size groups of 11-20, 21-30, and 31-40, respectively. 1 indicates that there is only one member; cannot form a group.

We classified the burst size into 3 groups: 11-20 (Romero, Karimac, and Lorelei), 21-30 (Blueyedbeauty, Izzy, Tribute, Wentworth), and 31-40 (Eddasa, Comrade). The results show that Tribute (BE) and Blueyedbeauty (BK1) having shared homologs as shown in Figure 3.13. Examples of those homologs include LysM-like endolysin (pham 8091), unknown function protein (pham 6800), DNA primase (pham 6755), etc. The total number of shared homologs is 48 genes out of 284 and 277 genes in Tribute and Blueyedbeauty, respectively. The other 2 groups do not have any similarity (Figure 3.11, 3.13, 3.14, 3.15, and 3.16).

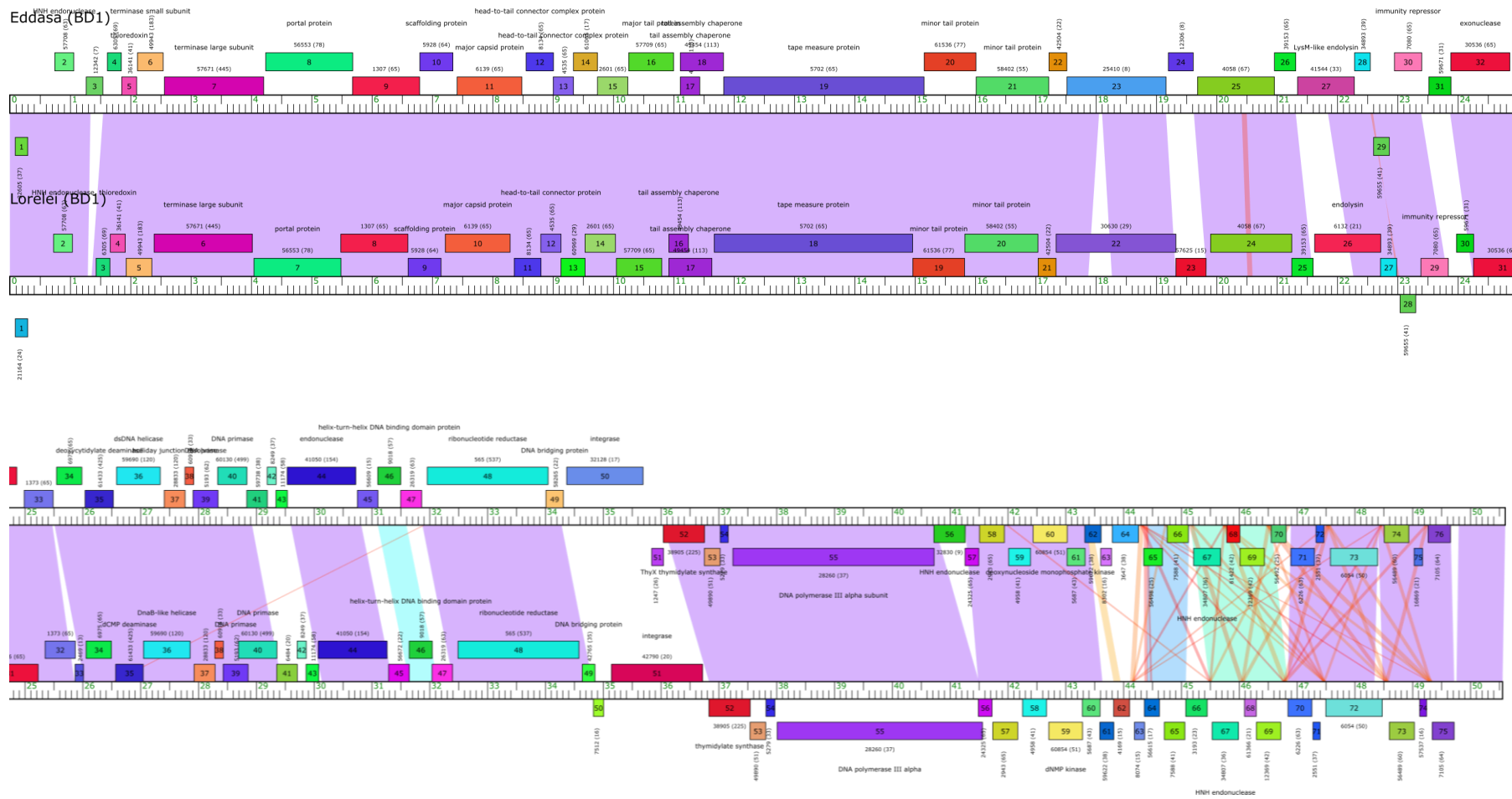


Figure 3.6 Comparison of Eddasa and Lorelei genome maps (41-60 minutes burst time): The two phages are in the same cluster, BD1 so they are closely related. Both phages share 52 homologs accounting to 68% and 69% similarities for Eddasa and Lorelei, respectively. There are 24 genes from Eddasa that do not appear in Lorelei genome (i.e. pham 12342 and 61065), and 23 genes from Lorelei that are not shared with Eddasa (i.e. pham 32605 and 30630). Purple shade indicates the most likely that the two genes are homolog (lowest E-value), while the red indicates the least likelihood (highest E-value). White area means no nucleotide similarity.

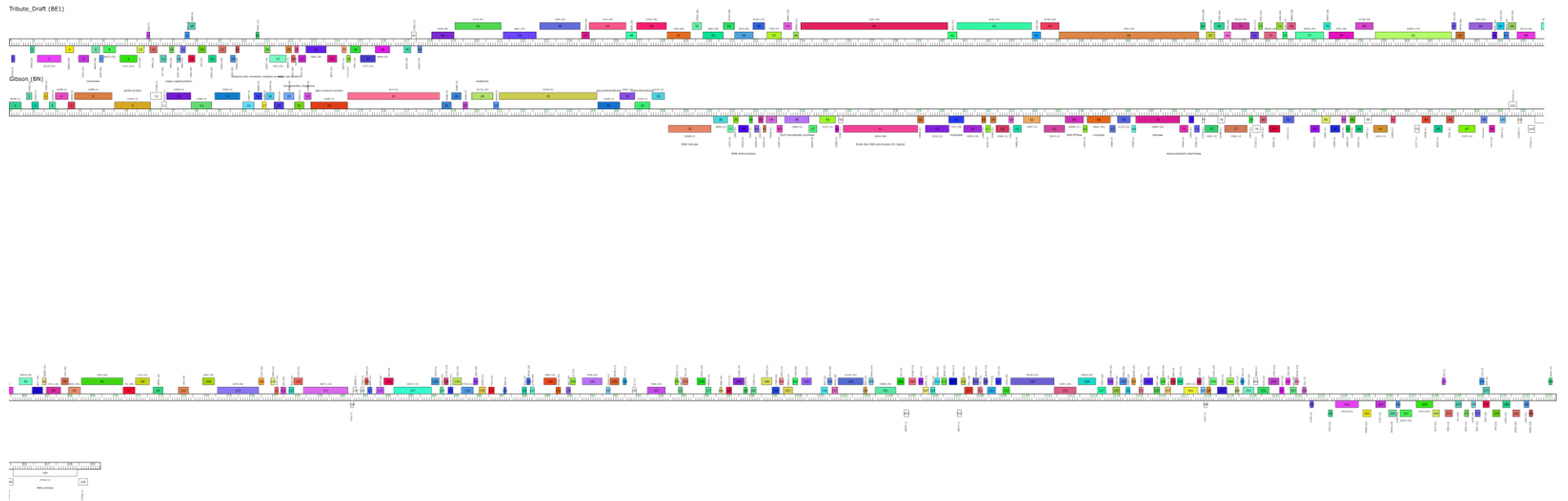


Figure 3.7 Comparison of Tribute and Gibson genome maps (101-120 minutes burst time): It is obvious that Tribute has a longer genome size (133.5 kbp) than Gibson's (69.4 kbp). They both come from the different clusters, BE1 for Tribute and BN for Gibson. They, therefore, do not have any shared homologs or purple regions.

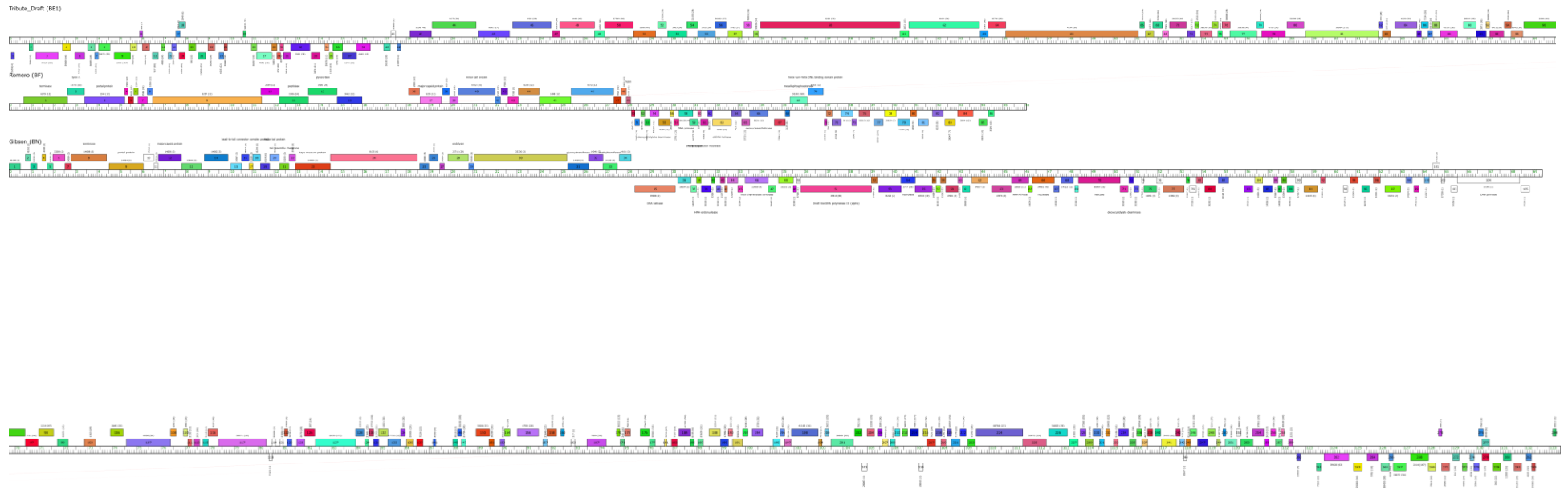


Figure 3.8 Comparison of Tribute, Romero, and Gibson genome maps (101-120 minutes burst time): Tribute has the longest genome size, followed by Gibson and Romero (46.1 kbp). There is no colored shading between Tribute and Romero, as well as Romero and Gibson. This means that there is no similarity between the two pairs.

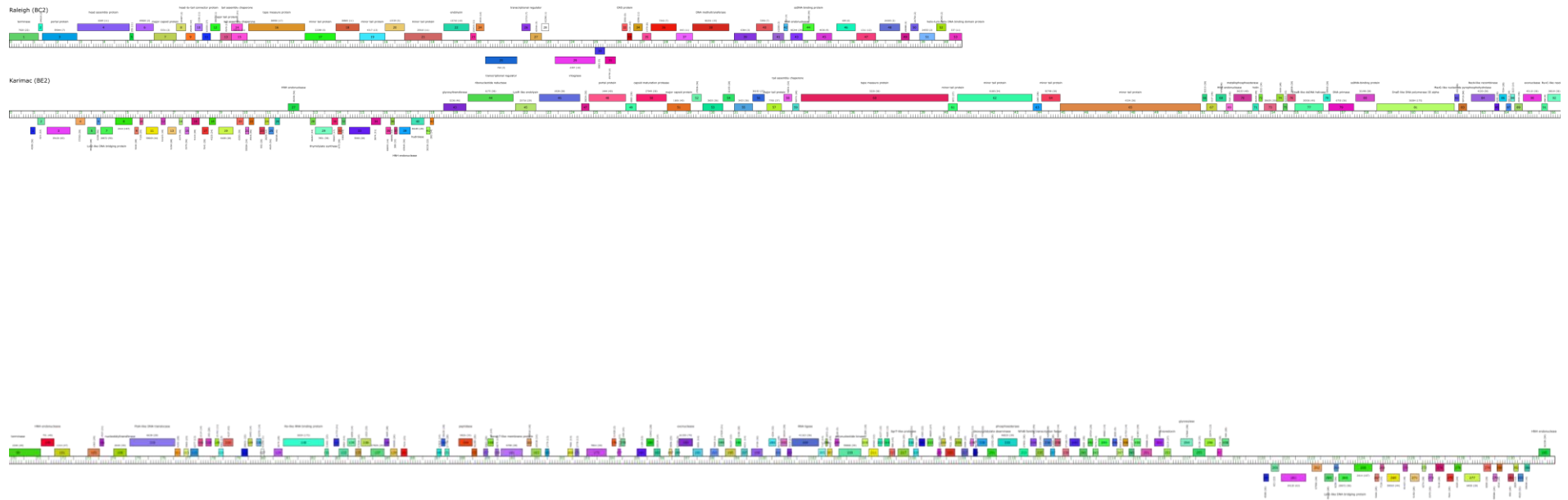


Figure 3.9 Comparison of Raleigh and Karimac genome maps (161-180 minutes burst time): Raleigh is the smallest phage (40.7 kbp) and Karimac is one of the largest phages (131.9 kbp) in this study. The genome map does not show any similarity.

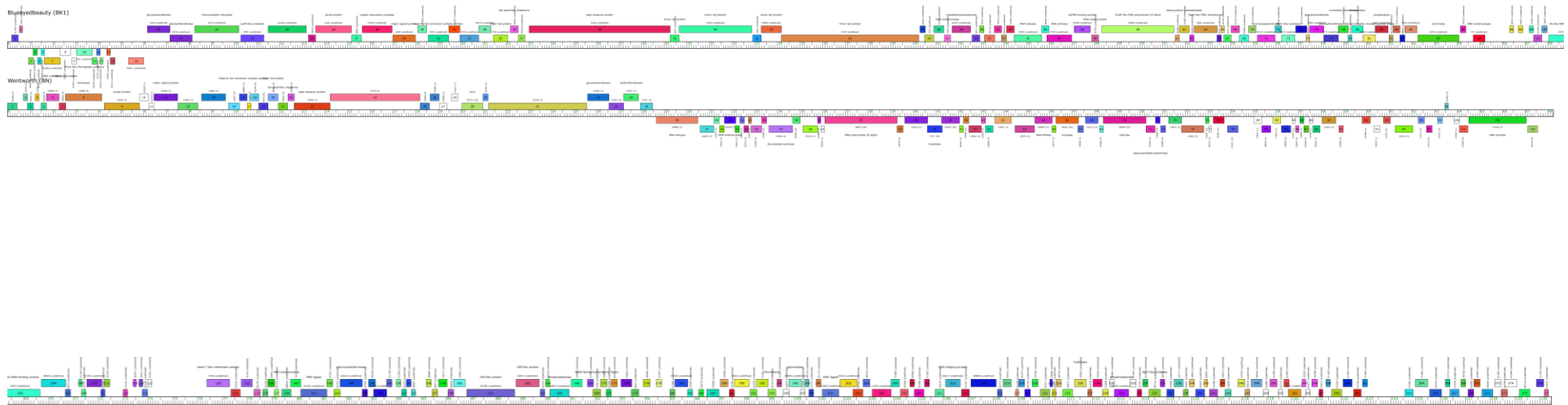


Figure 3.10 Comparison of Blueyedbeauty and Wentworth genome maps (281–300 minutes burst time): Blueyedbeauty has a larger genome size of 130.5 kbp, while Wentworth has a shorter genome size of 68.3 kbp. Both phages do not show any similarity according to the genome map.

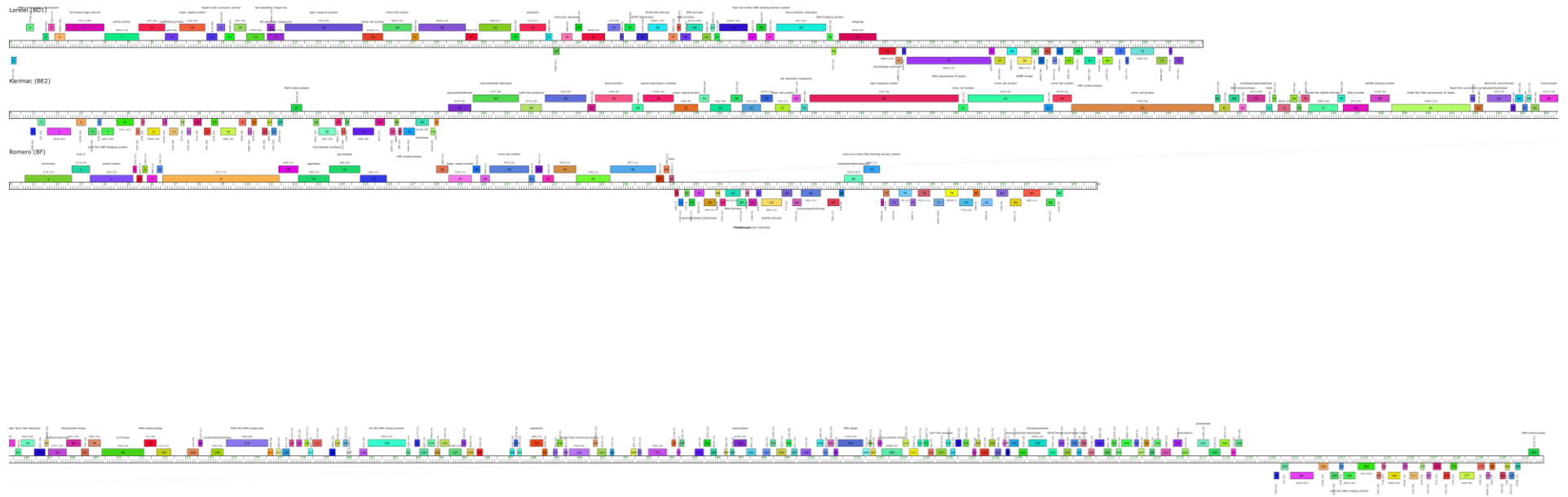


Figure 3.11 Comparison of Lorelei, Karimac, and Romero genome maps (11-20 burst size): Lorelei and Romero genome sizes are about the same, while Karimac has the longest one. Genome maps for Lorelei and Karimac, as well as ones for Karimac and Romero, do not show any similarity.

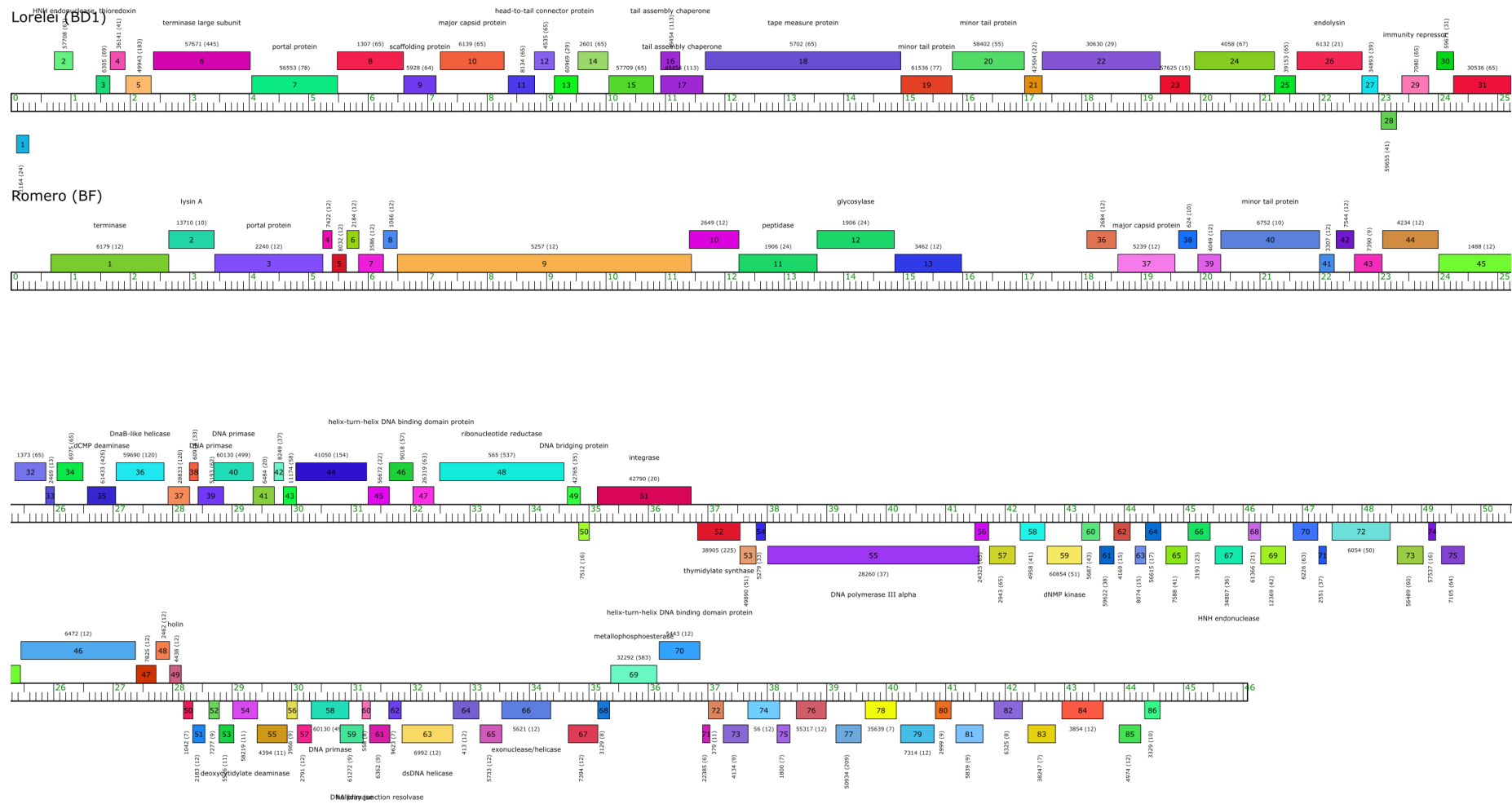


Figure 3.12 Comparison of Lorelei and Romero genome maps (11-20 burst size): No similarity has been found from the pair.

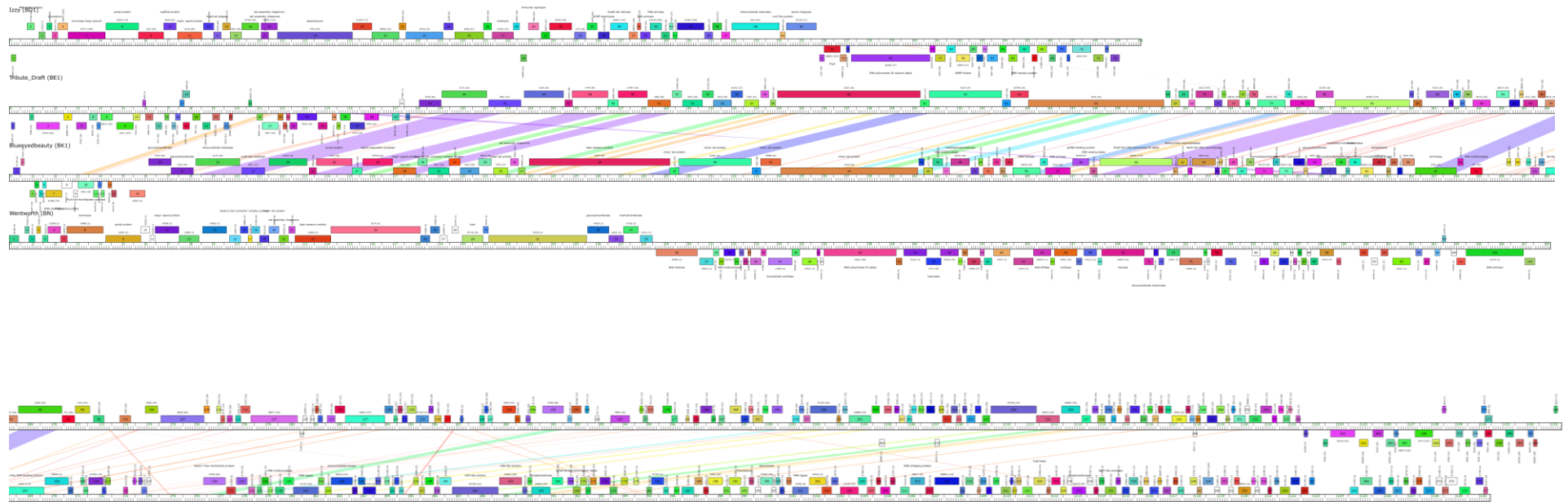


Figure 3.13 Comparison of Izzy, Tribute, Blueeyedbeauty, and Wentworth genome maps (21-30 burst size): There is no similarity found between Izzy and Tribute genomes or Blueeyedbeauty and Wentworth genomes. However, there are some homologs shared between Tribute (BE) and Blueeyedbeauty (BK1).

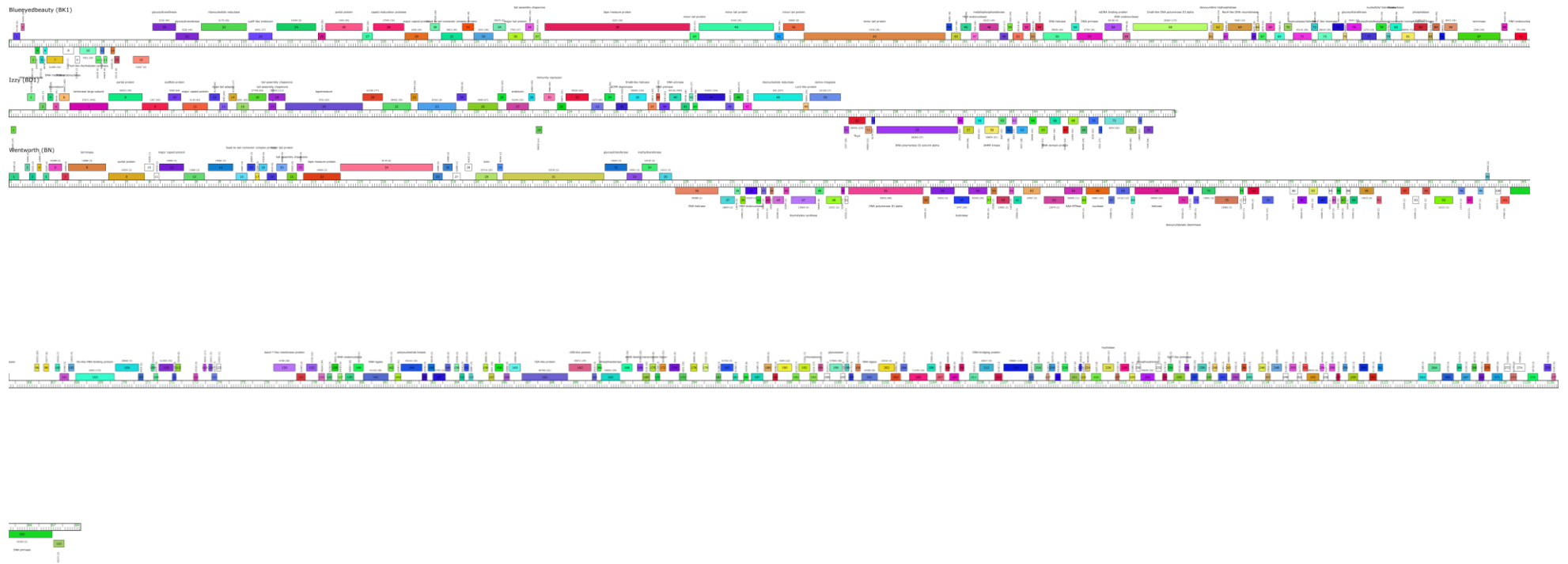


Figure 3.14 Comparison of Blueyedbeauty, Izzy, Wentworth genome maps (21-30 burst size): No similarity is found between Blueyedbeauty-Izzy and Izzy-Wentworth.

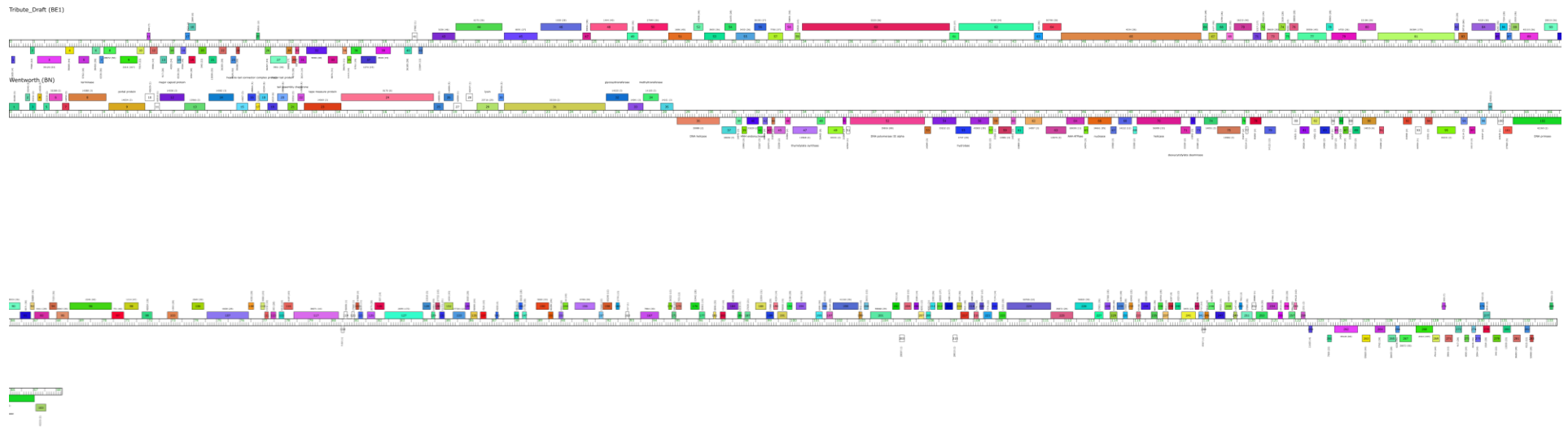


Figure 3.15 Comparison of Wentworth, and Tribute genome maps (21-30 burst size): No similarity found between the pair.



Figure 3.16 Comparison of Eddasa and Comrade genome maps (31-40 burst size): No similarity is found between the pair.

CHAPTER 4

DISCUSSION

4.1 *Streptomyces griseus* Growth and Doubling Time

It has been observed that bacterial growth rate influences phage growth rate (Nabergoj et al., 2017). In this study, we determined the host doubling time to estimate the minimum duration of phage growth. However, the problem of doubling time determination is that the host used in this study is filamentous.

Using a bacterial culture or spore to determine the cell number by spectrophotometry, the spread plating method, or viable cell count, are inaccurate. First, the cell number determined by the spectrophotometry could be inaccurate because the bacterial clumps are absorbing various wavelength, depending on the size of each clump. Second, one colony is not formed from one cell in the plating method, therefore, one colony-forming unit is not equal to one cell. Lastly, viable cell count is impossible because we cannot see an individual cell when they are clumpy. Nonetheless, Gilmour (1959) used *S. griseus* germinating spores to determine the cell number by using an electron microscope, which is prohibitive in time and resources for this study. This issue also leads to difficulty in the cell count for the one-step growth studies, because the number of infected cells must be used to determine burst size.

For this reason, we used the dry weight method to determine bacterial growth. The protocol was adapted from a doctoral dissertation (Hughes, L.E., 1998). Our results show a doubling time of 5 hours and 22 minutes which is approximately 3 hours faster than the same strain inoculated in the minimal liquid medium (Hughes, L.E., 1998). Surprisingly, this doubling time is very close to a growth result determined by plating method which showed a doubling time of 5 hours and 10 minutes (data not shown). However, the doubling time of 5

hours and 22 minutes was used as a guideline for the incubation period during the one-step growth studies of the 16 phages.

4.2 Phage Growth Protocol: A Filamentous Host Poses the Most Difficult Challenge

As mentioned above, one important challenge of this study is the filamentous host which leads to several complications. (1) It is difficult to determine the cell number used to infect with phages. (2) It is very likely that multiple infections can occur throughout the time course; free phages would come from the unwashed phages at $T=0$ as well as the released progenies after the burst time. (3) Phages could be hidden in the hyphae/mycelia resulting in an inaccurate infected cell determination. (4) When multiple infections occur, it is difficult to conclude an accurate burst size. (5) We successfully killed all the cells by using extremely high MOI (data not shown); however, it was only one time in about 50 times of infection. Moreover, using the high MOI is not a good idea because the higher the MOI, the bigger the burst size (Gadagkar et al., 1980). It is also possible that the phages would enter the lysogenic cycle when using higher MOI (Thakkar et al., 2018).

(1) It would be impossible to get an accurate number of the filamentous host cells by colony count, spectrophotometry, or viable cell count. We also cannot apply the idea of bacterial dry weight because we need to use the living cells for the phage growth study. In order to overcome this problem, we broke down the hyphae and mycelia in a diluted bacterial culture by a low-power sonication (4 Watts); then the single cells and short branches were isolated and collected by low-speed centrifugation (300 x g, 3 minutes). The method is modified from one of Dowding's publications (1973). Although there were some short hyphae mixed with the single cells, we accepted it if we were able to count the living cells under a microscope.

(2) Multiple infections are another difficulty for one-step growth. According to Hyman et al. (2009), the multiple infections for single-cell hosts (i.e. *E.coli*) can be prevented by dilution because the free phages (or liberated progenies) would not be able to meet and infect the new host cells (Figure 4.1). However, when a filamentous host is used, the free phages can infect the adjacent cells easily, leading to multiple infections (Figure 4.2). This also took place in this study, even if we already broke the hyphae. Furthermore, during the experiment, we always observed free phages at T=0 (Table S2) which were not washed away. It is possible that they infected the cells later and/or were hidden in the remaining hyphae.

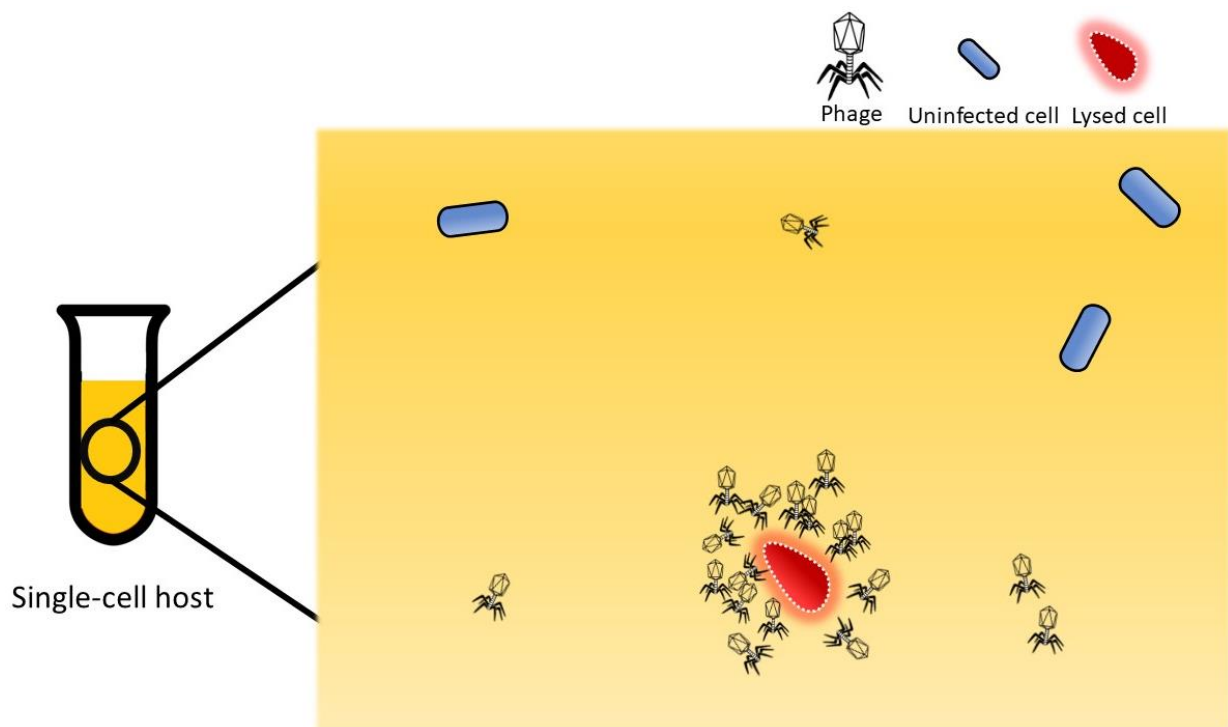


Figure 4.1 Progenies from a single-cell host in the diluted biological system: The liberated phages cannot meet the host cell. This prevents the phages from entering the second cycle.

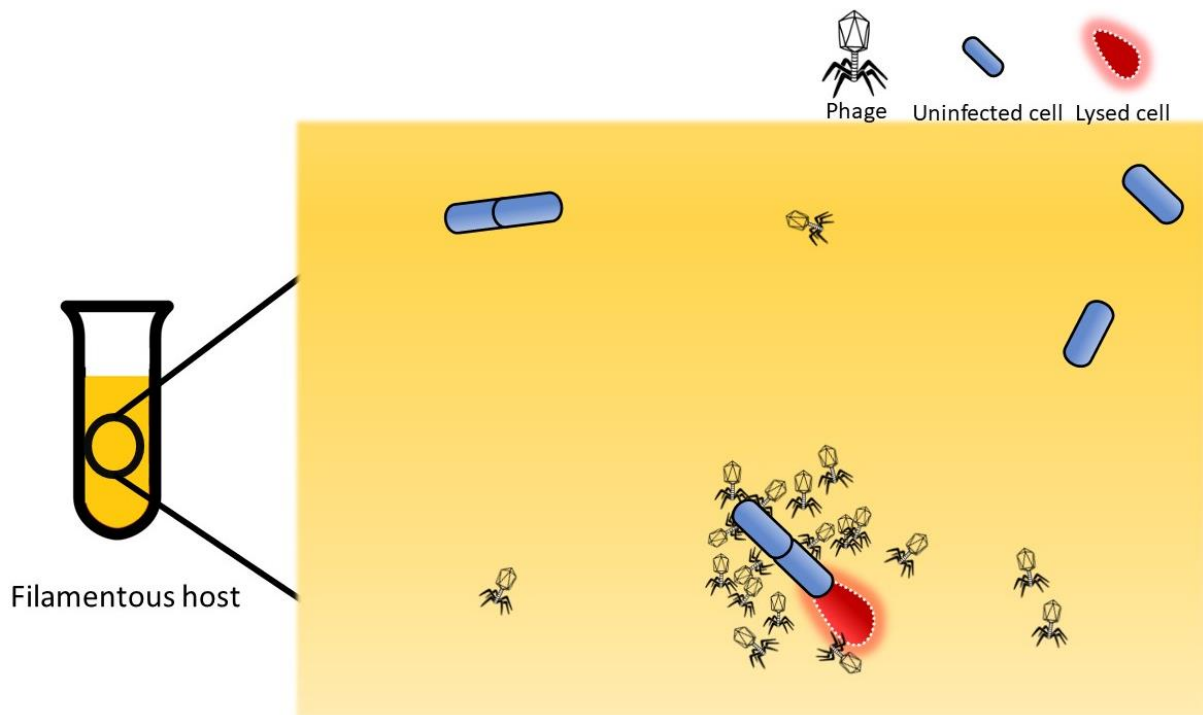


Figure 4.2 Progenies from a filamentous host in the diluted biological system: In filamentous cell-phage sample, progenies immediately adsorb to the adjacent cells after they successfully lyse the former cell. This could happen even though the suspension is diluted.

(3) If the free phages were hidden in the hyphae, the infected cell number might not be accurate because when we measure the free phages at $T=0$, we filter the cells out. If there are some phages hidden in the hyphae and get filtered out, together with the cells, the number of infected cells would be higher (Figure 4.3). The more infected cells determined, the less burst size observed.

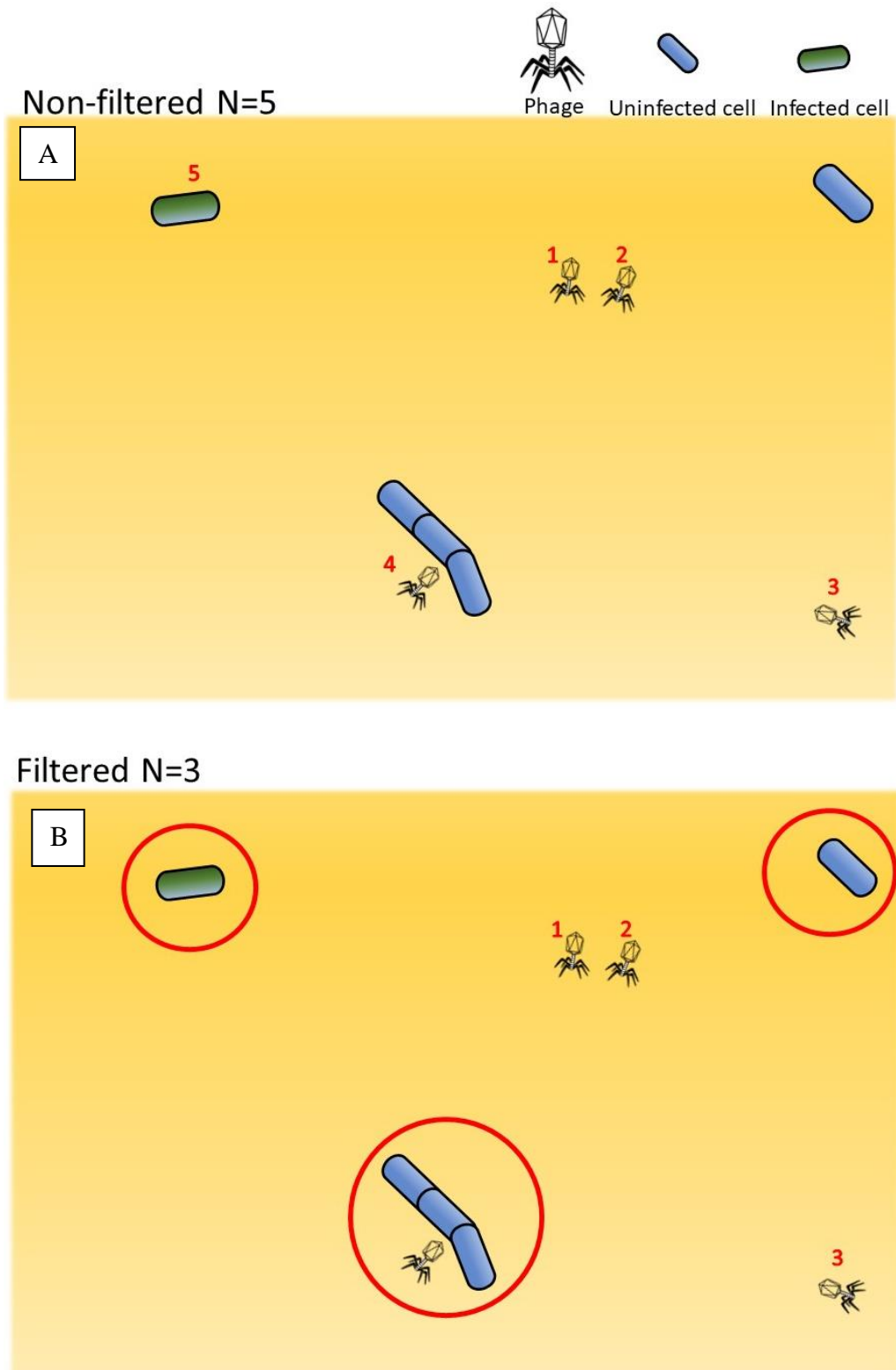


Figure 4.3 Inaccurate infected cell number: Infected cell number could be inaccurate if there are some phages hidden in the hyphae of the host. (A) and (B) represent the same sample at $T=0$. The system shows only 1 infected cell. (A) Collected sample without filtering shows 4 phages and 1 infected cell creating 5 plaques ($N=5$) on agar. (B) The red circles show the cells and phage that are filtered away. Therefore, there will be only 3 phages that can form 3 plaques ($N=3$). This leads to the inaccurate infected cell number of 2 cells ($N=2$).

(4) It is also tricky to determine burst size when multiple infections occur. According to the graphs in Figure 3.3, burst sizes are reduced after reaching the summit. This is because some released progenies adsorb or infect the new cells. It could be possible that if we were able to avoid the later cycles completely, the burst size would be bigger than what we observed. However, the best way we could do this is to pick the peak as a representative of the burst size.

Other than the filamentous host problems, the idea of using a low MOI of 0.1 does not seem to fit with this study. According to Hyman (2009), using the low MOI of 0.1 is beneficial to limit one phage particle to infect one cell. This can be explained by Poisson's model shown below:

$$f_{m>1}/f_{m>0} = (1 - e^{-M}(1 + M))/(1 - e^{-M})$$

- f_m is the probability of adsorption by the m number of phages.
- $f_{m>1}$ is the probability of multiple phages/cell.
- $f_{m>0}$ represents the probability of infection.
- M is MOI

Thus, the ratio reflects the proportion of cells infected by more than one phage. The lower the ratio is, the higher the number of cells infected by one phage. If we plug in $M=0.1$, the probability that the cells will be infected by more than one phage particle is 5%, meaning that 95% of the cell population is infected by one phage particle (one phage/cell). The reason why we focus on phage number infecting one cell is that burst size could be larger when the higher MOI is employed.

As mentioned earlier, we failed to obtain phages reasonable burst size with an MOI of 0.1 after more than 10 infections of BryanRecycles, Izzy, and Lorelei. We, therefore, increased and maintained the MOI of 0.5 for the entire study.

4.3 Burst Times and Burst Sizes for the 16 Phages

Gilmour (1979) has reported a burst time of 120 minutes for *S. griseus* phage 514-3. This burst time correlates with the ones reported in this study which vary from 45-405 minutes (see Table 3.1). The burst times for *S. griseus* phages are quite long compared to phages infecting other species (Table 1.2). The shortest burst time of 12.2 minutes, for the gathered data in Table 1.2, belongs to *E. coli* phage ϕ X174, while the longest one (120 minutes) belongs to phage 514-3. This indicates that our phages (i.e. Percastrophe, ToriToki, Wentworth) have even longer burst times than the reported ones. Only two of them, Romero and Gibson, show the same burst time as phage 514-3.

Nevertheless, the burst times for the entire population (shown in Table 3.1) are not correlated with either genome size or GC content. It is understandable for genome size to not be correlated with burst times because the replication rate would not depend solely on the length of the genome. There would be many factors such as holin and lysin gene expression levels, phage assembly rate, etc. Once the phage infects its host, there would be many different intracellular activities occurring at different times. According to the lytic-lysogenic decision of phage λ , the phage makes a decision to enter the lysogenic cycle when CII protein accumulation reaches the threshold. However, before the *cII* is expressed, there are several proteins, such as CRO, N, Q, CI, and CIII, working in a cascade sequence (Court et al., 2007). Although these proteins are found in lambda phage, they are not necessarily found in the phages in this study. Among the 16 phages in this study, there are numerous genes with no known function that could have activities that may affect burst time.

Table 4.1

Burst sizes from various host organisms (retrieved from <http://book.bionumbers.org/how-many-virions-result-from-a-single-viral-infection/>).

virus	host cell used	burst size	BNID
multicellular host			
influenza A & B	chicken egg cells	500-1,000	101590
influenza A	MDCK cell line	1,000-10,000	101605
HIV	<i>H. sapiens</i> memory T cells	1,000-3,000	105872
SIV (model for HIV)	<i>R. macaque</i> T cells	40,000-60,000	102377
prokaryotic host			
cyanomyovirus S-PM2	<i>Synechococcus WH7803</i>	40	104841
podovirus P60	<i>Synechococcus WH7803</i>	80	104842
cyanomyovirus MA-LMM01	<i>M. aeruginosa</i>	50-120	103247
bacteriophage S1	<i>Stenotrophomonas sp.</i>	80	104855
bacteriophage S3	<i>Stenotrophomonas sp.</i>	100	104852
phi EF24C	<i>E. faecalis</i>	100	104857
bacteriophage Lambda	<i>E. coli</i>	150	105025
bacteriophage T1 to T7	<i>E. coli</i>	100-300	105870
bacteriophage MS2	<i>E. coli</i>	5,000-10,000	109050

As shown in Table 4.1, bacteriophage burst sizes can be varied from 40 to 10,000, while Table 1.2 shows that burst sizes could be as small as 21.7, data obtained from *P. aeruginosa* phage HWPB-2 (Lindberg et al., 2014). In this study, the smallest burst size we observed is 12 (Romero) and the greatest is 1500 (± 60) (Percastrophe); the large burst size indicates higher lytic activity. Additionally, the large one seems reasonable for the very long burst time of 420 (± 30) minutes. However, the smallest burst size may seem too small. There could be at least two explanations. First, there might be several phages hidden in the hyphae and they get filtered away. This results in a falsely high infected cells number causing a falsely small burst size since they are inversely proportional. Second, Romero actually has a small burst size, meaning that it does not have a high level of lytic activity. This could be confirmed by performing

technical replicates and further investigation. Another possible explanation for the small burst size could be the non-original host. It is possible that Romero has a different original host, so it cannot exploit resources from *S. griseus* effectively.

Although burst size is not correlated with genome length, cluster, and GC content, it is interesting that burst time and burst size do show a positive correlation for the entire population, the group of 40, and 50 kbp (Table 3.2).

Moreover, it is highly interesting that phages from the same cluster or subcluster do not have the same burst time and/or burst size. If we compare those genomes and explore the differences between them, it would be beneficial to study what regulates the two traits.

4.4 Conclusion

All 16 phages with genome sizes between 40.7 and 133.5 kbp show burst times of 45 to 405 minutes and burst sizes of 12 to 1500. There is no correlation between genome size and burst time ($R = -0.00718$, $P = 0.97894$) or genome size and burst size ($R = -0.32677$, $P = 0.21670$). However, these results are based on only the 16 *Streptomyces* phages. In order to obtain the clearer results, we would need to increase the sample size to increase the number and variation of phages. When we group the phages that have similar burst time/burst size and explored their genome maps, we found that Eddasa and Lorelei, from the same burst time group, share 52 homologs. The other groups do not show similarity. Moreover, Tribute and Blueeyedbeauty are the representatives of a burst size group that show similarity. They share 48 homologs with each other. Similar to burst time, the other groups of burst size do not show any similarity.

APPENDIX

SUPPLEMENTAL DATA

Table S1

Streptomyces griseus dry weight

Time (hour)	Pre weight (g)	Post weight (g)	<i>S. griseus</i> dry weight (mg)
0	0.0824	0.0858	3.4
4	0.0825	0.0859	3.4
8	0.0822	0.0912	9
12	0.0825	0.0986	16.1
16	0.0827	0.1005	17.8
20	0.0826	0.1044	21.8
24	0.0825	0.1062	23.7
28	0.0825	0.1087	26.2
32	0.0825	0.1102	27.7
36	0.0826	0.1127	30.1
40	0.0825	0.1153	32.8
44	0.0826	0.1158	33.2
48	0.0826	0.1194	36.8
52	0.0825	0.1204	37.9
57	0.0822	0.1205	38.3
60	0.0822	0.121	38.8
64	0.0823	0.1216	39.3
68	0.0824	0.122	39.6

Table S2

Data obtained from the 16 phages growth studies. FP indicates number of free phages.

No.	Phage name	Time points (minutes)	Infected cells +FP (pfu/10 mL)	Actual number of infected cells (cell/10mL)	FP at each time point (pfu/10 mL)	Number of progenies (pfu/10mL)	Burst time (minutes)	Burst size
1	BryanRecycles	0	4.067E+03	2.067E+03	2.000E+03	0.000E+00	210.00±0.00	192.58±96.77
		15			2.000E+03	0.000E+00		
		30			2.000E+03	0.000E+00		
		45			2.667E+03	6.670E+02		
		60			5.333E+03	3.330E+03		
		75			4.667E+03	2.670E+03		
		90			7.333E+03	5.330E+03		
		105			8.000E+03	6.000E+03		
		120			1.333E+04	1.130E+04		
		135			1.333E+04	1.130E+04		
		150			4.000E+04	3.800E+04		
		165			2.667E+04	2.470E+04		
		180			4.667E+04	4.470E+04		
		195			6.667E+04	6.470E+04		
		210			6.667E+04	6.470E+04		
		225			4.000E+05	3.980E+05		
		240			2.000E+05	1.980E+05		
		255			5.333E+05	5.310E+05		
		270			4.000E+05	3.980E+05		
		285			4.000E+05	3.980E+05		
		300			1.133E+06	1.130E+06		

(table continues)

No.	Phage name	Time points (minutes)	Infected cells +FP (pfu/10 mL)	Actual number of infected cells (cell/10mL)	FP at each time point (pfu/10 mL)	Number of progenies (pfu/10mL)	Burst time (minutes)	Burst size
2	Izzy	0	2.600E+04	6.000E+03	2.000E+04	0.000E+00	80.00±17.32	22.18±5.50
		30			2.000E+04	0.000E+00		
		60			4.000E+04	2.000E+04		
		75			4.000E+04	2.000E+04		
		90			4.000E+04	2.000E+04		
		105			1.500E+05	1.300E+05		
		120			1.200E+05	1.000E+05		
		180			1.600E+05	1.400E+05		
		240			1.600E+05	1.400E+05		
3	Blueyedbeauty	0	1.933E+04	1.867E+04	6.667E+02	0.000E+00	285.00±15.00	21.39±7.58
		60			6.667E+02	0.000E+00		
		120			4.000E+03	3.330E+03		
		150			6.667E+03	6.000E+03		
		180			4.000E+04	3.930E+04		
		195			4.000E+04	3.930E+04		
		210			6.000E+04	5.930E+04		
		225			8.667E+04	8.600E+04		
		240			5.333E+04	5.270E+04		
		255			1.267E+05	1.260E+05		
		270			9.333E+04	9.270E+04		
		285			8.000E+04	7.930E+04		
		300			2.000E+05	1.990E+05		
		315			4.000E+05	3.990E+05		
		330			4.000E+05	3.990E+05		
345			4.000E+05	3.990E+05				
360			3.333E+05	3.330E+05				
4	Comrade	0	2.500E+03	1.833E+03	6.667E+02	0.000E+00	150.00±0.00	32.36±0.00
		120			6.000E+03	5.333E+03		

(table continues)

No.	Phage name	Time points (minutes)	Infected cells +FP (pfu/10 mL)	Actual number of infected cells (cell/10mL)	FP at each time point (pfu/10 mL)	Number of progenies (pfu/10mL)	Burst time (minutes)	Burst size
	Comrade (<i>continues</i>)	135	2.500E+03	1.833E+03	4.000E+03	3.333E+03		
		150			5.333E+03	4.667E+03		
		165			2.667E+04	2.600E+04		
		180			6.000E+04	5.933E+04		
		240			2.667E+04	2.600E+04		
		300			4.000E+04	3.933E+04		
5	Gibson	0	1.707E+05	7.067E+04	1.000E+05	0.000E+00	120.00±0.00	55.19±0.00
		120			2.000E+05	1.000E+05		
		180			2.000E+06	1.900E+06		
		240			4.000E+06	3.900E+06		
		300			4.000E+06	3.900E+06		
6	Eddasa	0	6.300E+03	6.300E+03	0.000E+00	0.000E+00	45.00±0.00	31.75±0.00
		30			0.000E+00	0.000E+00		
		45			0.000E+00	0.000E+00		
		60			4.000E+04	4.000E+04		
		120			2.000E+05	2.000E+05		
		180			2.000E+05	2.000E+05		
		240			2.000E+05	2.000E+05		
7	Immanuel3	0	2.470E+04	4.700E+03	2.000E+04	0.000E+00	145.00±8.66	236.88±49.14
		60			3.333E+04	1.333E+04		
		120			2.000E+04	0.000E+00		
		135			1.333E+04	-6.667E+03		
		150			2.000E+05	1.800E+05		
		165			2.000E+05	1.800E+05		
		180			1.133E+06	1.113E+06		
		240			4.667E+05	4.467E+05		
300			1.267E+06	1.247E+06				
8	Karimac	0	2.333E+04	2.133E+04	2.000E+03	0.000E+00	180.00±0.00	17.09±3.34
		120			1.333E+04	1.133E+04		
		135			2.667E+04	2.467E+04		

(table continues)

No.	Phage name	Time points (minutes)	Infected cells +FP (pfu/10 mL)	Actual number of infected cells (cell/10mL)	FP at each time point (pfu/10 mL)	Number of progenies (pfu/10mL)	Burst time (minutes)	Burst size
	Karimac	150	2.333E+04	2.133E+04	1.133E+05	1.113E+05		
	(continues)	165			1.533E+05	1.513E+05		
		180			1.133E+05	1.113E+05		
		240			3.333E+05	3.313E+05		
		300			4.000E+05	3.980E+05		
9	Lorelei	0	3.570E+05	1.970E+05	1.600E+05	0.000E+00	80.00±17.32	19.15±9.73
		60			1.000E+06	8.400E+05		
		120			3.867E+06	3.707E+06		
		180			4.000E+06	3.840E+06		
		240			1.000E+07	9.840E+06		
10	NootNoot	0	2.067E+03	6.667E+01	2.000E+03	0.000E+00	245.00±8.66	270.00±173.21
		60			3.333E+03	1.330E+03		
		90			2.000E+03	0.000E+00		
		120			2.667E+03	6.670E+02		
		150			2.000E+03	0.000E+00		
		180			2.000E+03	0.000E+00		
		195			3.333E+03	1.330E+03		
		210			6.000E+03	4.000E+03		
		225			3.333E+03	1.330E+03		
		240			5.333E+03	3.330E+03		
		255			1.333E+04	1.130E+04		
		270			2.000E+04	1.800E+04		
		285			1.333E+04	1.130E+04		
		300			1.333E+04	1.130E+04		
		315			1.333E+04	1.130E+04		
11	Percastrophe	0	9.267E+03	1.933E+03	7.333E+03	0.000E+00	420.00±30.00	1513.45±59.73
		150			1.333E+04	6.000E+03		
		210			8.667E+03	1.330E+03		

(table continues)

No.	Phage name	Time points (minutes)	Infected cells +FP (pfu/10 mL)	Actual number of infected cells (cell/10mL)	FP at each time point (pfu/10 mL)	Number of progenies (pfu/10mL)	Burst time (minutes)	Burst size
	Percastrophe	270	9.267E+03	1.933E+03	1.467E+04	7.330E+03		
	<i>(continues)</i>	285			6.000E+04	5.270E+04		
		300			5.333E+04	4.600E+04		
		315			4.667E+04	3.930E+04		
		330			3.333E+04	2.600E+04		
		345			4.667E+04	3.930E+04		
		360			8.000E+04	7.270E+04		
		375			1.067E+05	9.930E+04		
		390			2.067E+05	1.990E+05		
		405			3.333E+05	3.260E+05		
		420			5.333E+05	5.260E+05		
		435			8.667E+05	8.590E+05		
		450			1.267E+06	1.260E+06		
		465			2.933E+06	2.930E+06		
		480			2.800E+06	2.790E+06		
		495			1.067E+06	1.060E+06		
		510			1.400E+06	1.390E+06		
		525			1.733E+06	1.730E+06		
		540			2.133E+06	2.130E+06		
12	Raleigh	0	2.600E+03	6.000E+02	2.000E+03	0.000E+00	185.00±8.66	996.67±577.35
		15			2.667E+03	6.670E+02		
		30			3.333E+03	1.330E+03		
		45			4.000E+03	2.000E+03		
		60			4.000E+03	2.000E+03		
		75			7.333E+03	5.330E+03		
		90			5.333E+03	3.330E+03		
		105			2.000E+04	1.800E+04		
		120			1.333E+04	1.130E+04		

(table continues)

No.	Phage name	Time points (minutes)	Infected cells +FP (pfu/10 mL)	Actual number of infected cells (cell/10mL)	FP at each time point (pfu/10 mL)	Number of progenies (pfu/10mL)	Burst time (minutes)	Burst size
	Raleigh	135	2.600E+03	6.000E+02	1.333E+04	1.130E+04		
	<i>(continues)</i>	150			2.000E+04	1.800E+04		
		165			2.000E+04	1.800E+04		
		180			2.000E+04	1.800E+04		
		195			2.667E+05	2.650E+05		
		210			5.333E+05	5.310E+05		
		225			1.333E+05	1.310E+05		
		240			1.333E+05	1.310E+05		
		255			6.667E+04	6.470E+04		
		270			6.667E+04	6.470E+04		
		285			1.333E+05	1.310E+05		
13	Romero	0	3.500E+03	1.500E+03	2.000E+03	0.000E+00	120.00±0.00	12.00±0.00
		120			6.000E+03	4.000E+03		
		135			2.000E+04	1.800E+04		
		150			2.000E+04	1.800E+04		
		165			2.000E+04	1.800E+04		
		180			2.000E+04	1.800E+04		
		240			2.000E+04	1.800E+04		
		300			2.000E+04	1.800E+04		
14	ToriToki	0	6.667E+02	6.667E+02	0.000E+00	0.000E+00	345.00±0.00	190.00±62.45
		150			0.000E+00	0.000E+00		
		270			0.000E+00	0.000E+00		
		300			0.000E+00	0.000E+00		
		315			0.000E+00	0.000E+00		
		330			0.000E+00	0.000E+00		
		345			0.000E+00	0.000E+00		
		360			1.267E+05	1.267E+05		
		420			1.067E+05	1.067E+05		

(table continues)

No.	Phage name	Time points (minutes)	Infected cells +FP (pfu/10 mL)	Actual number of infected cells (cell/10mL)	FP at each time point (pfu/10 mL)	Number of progenies (pfu/10mL)	Burst time (minutes)	Burst size
15	Tribute	0	1.740E+04	1.540E+04	2.000E+03	0.000E+00	105.00±0.00	25.84±0.00
		120			6.000E+03	4.000E+03		
		135			2.000E+03	0.000E+00		
		150			2.000E+03	0.000E+00		
		165			2.000E+04	1.800E+04		
		180			1.000E+05	9.800E+04		
		240			4.000E+05	3.980E+05		
		300			1.400E+05	1.380E+05		
16	Wentworth	0	6.667E+04	5.067E+04	1.600E+04	0.000E+00	275.00±31.22	29.95±7.44
		120			9.333E+03	-6.670E+03		
		135			1.333E+04	-2.670E+03		
		150			2.200E+04	6.000E+03		
		165			1.000E+05	8.400E+04		
		180			6.000E+04	4.400E+04		
		195			1.800E+05	1.640E+05		
		210			2.333E+05	2.170E+05		
		225			2.267E+05	2.110E+05		
		240			4.000E+05	3.840E+05		

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