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Characterization of Growth for the Subcluster B5 Mycobacteriophage Donny Using Optical Density Measurements

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Hannah L. Coltrain
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Abstract

The mycobacteriophage Donny was discovered in Dahlonega, GA in 2016 as part of the SEA-PHAGES Program and is currently only one of seven verified phages in subcluster B5. Through various protocols for phage DNA purification and amplification, gene expression can be observed during lysis of its host, *Mycobacterium smegmatis* using real time PCR. Bacteriophages provide inherently novel subjects for genetic analyses as they are a highly diverse and ubiquitous group. There is little published research on phage from Cluster B which are a large yet genetically distinct group compared to other clusters or singletons. In order to begin analyzing gene expression during the phage life cycle, observation of Donny’s unique burst time is required so that optimal time frames can be selected for RNA extraction. Using absorbance data obtained from Optical Density (OD) measurements at a wavelength of 600nm, a potential time frame for RNA extraction during early, middle, and late infection was determined. This project contributes to the overall goal of characterizing the genes expressed during Donny's lytic cycle using primers designed from sequence analysis of all 96 genes.

Introduction

The scope of the microbiological world is vast and diverse, including organisms that differ greatly in biochemical complexity, highly affect the macro world around them, and offer novel avenues for research and discovery. Representing the majority of life on Earth, bacteriophage, also known as simply “phage,” are viruses that are capable of infecting bacteria (Hatfull 2011). They are a major faction of the microbiology discipline that present far-reaching research applications that include evolutionary studies on their genetic diversity, biochemical processes related to virus-host interactions, and viral life cycles. The potential for gene function novelty is high within the large phage genome pool as most phage do not share genetic similarities outside of their closely-
related groups (Hatfull 2011). This study observes a virulent mycobacteriophage named Donny by its detector that infects the host *Mycobacterium smegmatis* and creates small plaques. First, however, it is important to understand the basics of bacteriophage as novel, ubiquitous “organisms,” and the methods by which they are characterized.

The general morphology of a bacteriophage includes a proteinaceous capsid, or “head,” that contains the genetic material which is relatively certain to be double-stranded DNA (Hatfull 2014). Genome size varies greatly. With mycobacteriophages, the capsid is attached to the tail, which also varies greatly in length, and aids in the efficient delivery of phage DNA into the host. Of the three morphotypes – *Siphoviridae*, *Myoviridae*, and *Podoviridae* – describing tail morphology, mycobacteria seem to only have either the long, flexible, noncontractile tail of the *Siphoviridae*, or the long, contractile tails of the *Myoviridae*. The tail plays a key role in host recognition and infection ability (Veesler 2011). Overall, phage are organized into Clusters that are named both alphabetically and numerically, such as Cluster D containing Subcluster D2. These groupings rely mostly on genomic characterization, where the nucleotide sequence similarity must span over 50% of another phage’s genome (Hatfull 2014). This system is helpful in depicting a baseline relatedness between sequenced phage, but it does not reveal defined evolutionary relationships when taking into account the mosaicism of phage genomes in which segments of code have distinct evolutionary histories and can be swapped horizontally between phage. The heterogeneity between groups can also be substantial (Hatfull 2014). Bioinformatic tools such as Phamerator can be used to visually compare sequenced genomes of various phage, and depict the mosaicism of protein-coding sequences (Cresawn et al 2011).

Mycobacteriophages are capable of specifically infecting mycobacterial hosts which are a distinct group of bacteria with characteristic cell walls containing mycolic acid, a long glycolipid that contributes to this taxon’s wax-like outer layer and clumpy growth pattern. Notable members
of this group include *M. smegmatis* and *M. tuberculosis*. The former is an optimal host for mycobacteriophage study because of its relatively fast life cycle of only 2-3 days, lack of pathogenicity, and ubiquity within soil and water. Many of the known mycobacteriophages infect various strains of *M. smegmatis*, making this host ideal for characterizing phage (Hatfull 2014). Phage can be enumerated via a plaque assay procedure using a collected lysate from an isolated environmental sample. Plaques can be seen on plates as cleared, circular areas on a bacterial lawn of growth; occurring from multiple rounds of infection, this clearing comes from the subsequent destruction of the host by, theoretically, a single phage particle lineage. Counting plaques can be used to calculate the titer, in units of plaque forming units per milliliter (pfu/mL) of a lysate, a liquid culture of the phage. The cycle of infection varies between phages, but can be either lytic, as with virulent phage that goes through a definite cycle of adsorption, genome replication, phage assembly, and phage release, or lysogenic, in which a temperate phage is known to make a lysogen that houses the phage genes within its own genome (Fig. 1). The timing of a phage replication cycle varies, with three general phases appearing similarly to a bacterial growth curve. Firstly, the latent phase represents the period between phage adsorption to and lysis of the host, visualized by an increase or plateau in host growth and absence of plaques (Abedon 1989). Secondly, the rise period is analogous to the log phase of bacterial growth, and occurs when newly assembled phage virions burst out of the host, visualized by a rise in the pfu/mL on a plaque assay. Finally, the plateau phase is reached when cell lysis is complete and the amount of plaques remains relatively constant over time. One-step growth curves can be generated over time through multiple plaque assay procedures to detect a change in pfu/mL.
Donny is a Cluster B, Subcluster B5, virulent mycobacteriophage with siphoviridal morphology, isolated from an enriched soil sample on the University of North Georgia campus in 2016 as part of the international Science Education Alliance Phage Hunters Advancing Genomic and Evolutionary Sciences (SEA-PHAGES) program. It was isolated in the host \textit{M. smegmatis mc}^2155 and creates small (0.1cm) clear plaques (Fig. 2), indicative of a slow adsorption rate. Its circular genome is comprised of 96 genes that include both forward and reversely transcribed promoters. Upon aligning Donny’s genome with other Subcluster B5 phage Acadian, Rich, Baee, Phelemich, and Reprobate using the NCBI Standard Blast tool, several nucleotide sequences of high-percentage similarities can be seen. Donny seems to be most closely related to Acadian and Baee, but also contains novel “orphan” genes such as gene 51 and gene 70 that share less or no homology (Fig.3). Sequence alignment data is useful in seeing the heterogeneity within a Subcluster as well as potentially novel transcription patterns. Using the online NCBI Primer Blast
tool along with Donny’s available FASTA sequences, primers were designed for each of Donny’s gene based off of proposed open reading frames (ORF) generated in GenBank. When designing primers, factors including primer length, GC content and melting temperature, annealing temperature, cross homology, and lack of runs and repeats were all considered. Several of Donny’s genes required use of reverse complement sequences to account for reversely transcribed ORF’s.

Figure 2. Characterization of mycobacteriophage Donny. (A) TEM picture of mycobacteriophage Donny, courtesy of UNG Honors Student Lauren Colston. The *Siphoviridae* morphology is readily pictured by the presence of a long tail, which can also be seen as slightly striated. The capsid diameter is about 60nm and tail length is about 286.67nm. (B) Donny creates small (~0.1 cm diameter) plaques on a lawn of *M. smegmatis mc²* 155, grown for 48 hours at 37°C.
Figure 3. Top DNA sequence alignment matches of Mycobacteriophage Donny genome using NCBI BLAST database. Each of Donny’s 96 genes were sequenced and run through a standard nucleotide BLAST search for optimal local alignments. The top five matches with an identity percentage >80% are graphically depicted for each gene and recorded in Table C. A and B: successive phage matches to Donny genes 1-48 and 49-96, respectively, with the first result located closest to the y-axis, and common mycobacterium phage alignments denoted by bar color, including phages Acadian (red), Rich (blue), Baee (green), Phelemich (gold), and Reprobe (black). Novel matches to phages AlanGrant (purple), Vicenzo (grey), and Adawi (orange) are also depicted. Bar sizes represent the identity percentage as given by the BLAST database and where 80% is the lowest possible identity relevant. C: Table of alignment identity percentage between each of Donny’s genes to other phages. Donny’s genes are grouped by those which are either forwards or reversely transcribed, and alignments with an Expect Value (E) of 0 are noted by bold-font numbers. Novel phage alignments are noted separately in the bottom right corner.
Currently, the literature characterizing any Subcluster B5 phage is few and far between; negligible publications exist that elucidate gene expression during lysis. For other phage, however, there are some experimental designs for isolation of total RNA from infected log-phase *M. smegmatis* using time point determinations using Optical Density (OD) data (Dedrick et al 2013, Payne et al 2009); OD data has also been used to determine burst time in *M. smegmatis* infected with Cluster A phage, D29 (Foddai et al 2009). This method of determining a burst time allowed for a less time-consuming procedure as well as more immediate acquisition of data given the 2-day growth time for plaque assays. Observing Donny’s burst time frame in *M. smegmatis mec²*155 is a novel experiment that is necessary before moving forward to RNA extraction. Future experiments on phage from this Cluster may benefit from any data collected.

**Experimental Design**

The burst time of a phage can be defined here as the period of time during which lysis of the host cells by the phage results in a decrease of absorbance using an $OD_{600}$ spectrophotometer. Lysis was monitored using $OD_{600}$ absorbance values with a known multiplicity of infection (MOI), a direct ratio of phage particles measured in pfu/mL to bacterial host cells measured in cfu/mL. Optical density has been used as a quick and indirect method for observing cell growth in liquid culture when paired with viable plate counting, but many limitations exist in its methodology, creating high potential for inaccuracy (Begot et al 1995, Wang et al 2010, Francois 2005).
Materials and Methods

Host Culture Propagation and Growth Curve. Ten microliters of strain *M. smegmatis mc²155* was inoculated from a frozen stock in 25 mL liquid Middlebrook 7H9 medium and grown for 2-3 days at 37°C and shaking at ~200 rpm. The resulting growth curve (Fig. 4) was referenced throughout the study in order to relate the amount of viable colony forming units (cfu) to absorbance at 600 nm.

![Growth Curve](image-url)

Figure 4. Average growth of host bacteria *M. smegmatis mc²155* in liquid culture. Optical Density (OD) absorbance values were recorded over several hours to assess the growth of the host bacteria after inoculation into Middlebrook 7H9 media. Data was taken in triplicate with each individual culture denoted in the legend by a different color and number, and *M. smegmatis* can be seen growing at similar rates. The “Average” series (blue) represents the overall average absorbance values at each time point, and also depicts the standard error of the mean (black bars) for each time point. The lag phase is from roughly 0-50 hours. The log phase of growth is estimated to extend between the large time frame of 50-70 hours before the stationary phase seen between 75-82 hours. The beginning of the death phase seems to occur around 82 hours or shortly thereafter.
Enumeration of *M. smegmatis* mc²155 by Viable Plate Counting. Host bacteria was grown on L-agar plates containing 50 ug/mL carbenicillin and 10 ug/mL cycloheximide to several different optical densities in order to relate cfu/mL to $OD_{600}$. 100 microliters of serially diluted *M. smegmatis* mc²155 were plated using 5mm glass beads and incubated for ~48 hrs at 37°C. Figure 5 provides an example of the difference in cfu/mL between dilutions. Figure 6 relates viable colony forming units to $OD_{600}$ values.

![Figure 5. Example viable plate count of *M. smegmatis*. Serial dilutions were performed on a liquid culture grown to a known OD value. Reliable amounts of colony forming units lie between 30-300 cfu.](image)

![Figure 6. Amount of viable *M. smegmatis* in liquid culture based on Optical Density. Several viable plate counts of host bacteria were performed from diluted aliquots that were grown to the OD specified on the horizontal axis. 100 μL aliquots were plated and incubated for ~48 hours at 37°C. As expected, there seems to be an overall positive correlation between absorbance and the amount of viable bacteria in liquid culture. OD values were chosen based on experimental grounds from cited literature that infected host cells at different stages of growth. Note the lack of error bars due to the fact that these data were not performed in triplicate. More data is needed to relate OD values to cfu/mL with confidence.](image)
Plaque Assay for High Titer of Donny Lysate. Plaque assays and titer calculations were performed according to the 2016 SEA-PHAGES Phage Discovery Guide for a Full Plate Titer. More than one assay was performed to confirm the pfu/mL of each new lysate. 10 uL of serially diluted lysate was adsorbed with 250 uL of *M. smegmatis* mc2155 for 15-30 minutes at room temperature before addition of 100 mM CaCl2 and Middlebrook 7H9 media. Samples were then mixed with molten 1X Middlebrook Top Agar and poured onto L-agar plates prepared as previously mentioned and left to set. Plates were incubated overnight at 37℃. A non-infected control was also plated and incubated each time. Plaques produced were used to calculate the titer of the lysate (Fig. 7). Propagation of Donny’s lysate was done by flooding webbed plates with 8 mL of Middlebrook 7H9 media and allowing them to pool for 2-4 hours at room temperature before filtration using a sterile 0.22 μm filter into a sterile conical tube. A new titer was calculated for every new lysate. Lysates were stored at 4℃ for use in later experiments.

Figure 7. Example full titer plaque assay for mycobacteriophage Donny. Serial dilutions of a lysate were plated according to Full Plate Titer procedure of the SEA-PHAGES Discovery Guide. A “webbed plate” of growth, characterized by a high number of plaques across a bacterial lawn that creates a web-like appearance, is seen as a 10^-4 dilution of the lysate. The 10^-6 plate represents a countable number of plaque forming units that can be used to calculate the titer, a measure of concentration of phage particles within the liquid lysate. The titer of this lysate was calculated to be 5.0 * 10^9 pfu/mL.

Burst Time Determination Using **OD**$_{600}$. In accordance with a previous experiment by Payne et al (2009), the **OD**$_{600}$ value of 0.25 was set for the infection of a known amount of host bacteria (in cfu/mL) with a known amount of bacteriophage (in pfu/mL) to coincide with a
predetermined MOI. An initial experiment tracking the OD of infected host culture at an OD around 1.0, per Dedrick et al (2013), and with the MOI’s of 1, 5, and 10 was performed. Multiple experiments were performed that manipulated the time of each reading after infection, the MOI used, and the time of adsorption. A single liquid culture of host cells, plus a control specimen, were grown as described and diluted to an $OD_{600}$ around 0.25. After infection with Donny corresponding to the desired MOI, absorbance readings of 1 mL samples were taken using a Spectronic Genesys 2 spectrophotometer. When done in triplicate, the original culture was aliquoted into separate 15 mL Falcon tubes before each were inoculated with phage. The MOI was calculated using the direct amounts of phage (pfu/mL) to bacteria (cfu/mL). Phage were left to adsorb at room temperature and without shaking.

**Results**

**Determination of suitable MOI for M. smegmatis mc² 155 at OD around 1.0.** Infected host cultures of different MOI’s of 1, 5, and 10, were evaluated after a 30 min adsorption time using absorbance values to evaluate lysis. In addition to OD data, direct plating of 260 μL of the infected culture was plated onto L-agar using the previously described plaque assay procedure. In error, a control culture was not evaluated for this data set. Data is summarized in Figure 8a-b. OD values steadily rose in the cultures infected with MOI’s of 5 or 10; a decrease in OD can be seen with the MOI of 1 between roughly 75 and 135 minutes after the 30 min adsorption period. However, few conclusions may be drawn due to the lack of a control measurement. Plating at each time point yielded inconclusive results as patterns of potential clearing of *M. smegmatis mc² 155* is unclear when not coupled with a control sample.
Determination of burst time using varied MOI and standard adsorption time. *M. smegmatis mc²155* was infected with Donny, according to the methods previously described, at MOI’s of 0.01, 0.1, and 1, and the OD values were recorded at varied time intervals after a 30 min adsorption at room temperature. Data was collected in two sets which differ in the day that the experiment was performed as well as the extent of time the experiment lasted. Graphed results are summarized in Figures 9(a-e). A decrease in OD of some amount was consistently recorded between a 75-90 minute time frame, though a confound between data sets exists as both sets represented in 9a and 9b showed increases in OD over time after infection, while cultures represented in 9c and 9d showed a steady decline. Potential error within the data, especially pertaining to the earlier sets are considered at length in the Discussion. The later two yielded results consistent with other published experiments using OD data, in that the infected samples exhibit a sinusoidal pattern of increased growth followed by a peak, potentially representing the burst of host cells, and subsequent steady decline in OD.
Figure 9a. **Absorbance of infected liquid cultures at different multiplicity of infection and with different initial OD values.** All host cultures were grown to an OD around 0.25 according to a similar procedure by Payne et al (2009). Absorbance of each culture was recorded every 30 min for four hours before recording at 15 min intervals under the assumption that an overall decrease in bacterial growth should be seen. A general gradual increase in OD over time is seen for all cultures, though it is difficult to visualize a difference to the control because of a difference in initial OD’s prior to infection (not pictured). Before the 30 min adsorption period, OD values were 0.229 for the control, 0.240 for the MOI of 0.1, and 0.320 for the MOI of 1. This discrepancy was accounted for in later experiments when cultures were first diluted to the desired OD before the addition of phage.

Figure 9b. **Absorbance of infected liquid host cultures at different multiplicity of infection recorded at hour intervals.** OD was monitored for ten hours to observe if an overall drop in OD could be seen over an even longer period of time. These data show that all cultures increased in OD, acting against the prediction that a burst may be seen by a sudden, and then sustained, decrease in OD, and subsequent continuation over a longer time period. Phage-infected cultures continued to have increased growth from the first recorded OD after a 30 min adsorption period. Compared to the control, the initial OD’s were both lower for infected cultures after the first 30 minutes.
Figure 9c. **Absorbance of initially diluted cultures infected according to three different MOI’s over time.** All cultures were diluted with Middlebrook 7H9 media to an OD of 0.25 prior to phage addition. 1 mL OD measurements were taken after a 30 min adsorption time at room temperature every 15 min for two hours, with two additional measurements at the third and fourth hours. For the lowest MOI of 0.01, a peak OD is seen at 60 and 75 minutes before starting to decline at the next 15 minute interval. For the MOI of 0.1, a similar trend is seen, though the fitness of data to the trendline is greatly decreased. With an MOI of 1, a trend is even less clear, with the peak OD occurring at 90 min before dropping off. OD of the control stayed above the variable cultures with the exception of the 60 minute time period; this dramatic difference may be a result of little vortexing of the sample before reading OD. Polynomial trendlines depict the data best because of the sigmoidal nature of microbe growth. The $R^2$ value for each trendline is noted next in the legend next to each variable.
Figure 9d. **Replication of absorbance trends of initially diluted cultures according to three different MOI’s over time.** Replicating the same procedure used for Figure 9c, 1 mL OD measurements were taken after a 30 min adsorption time at room temperature every 15 min but for two hours only. The $R^2$ value for each polynomial trendline is noted in the legend. The control’s OD remained above the three other variables, consistently increasing over time. A closer look at the different MOI’s is seen in Figure 9e.

Figure 9e. **Absorbance data for infected cultures only from Figure 9d.** A peak OD is seen at 75 minutes before dropping at the next measured time point. Data reveals a pattern of growth consistent with predicted bacteriophage behavior and the lytic cycle, with the host’s growth seen to increase, according to OD, before lysis occurs. The hundredfold difference between the MOI’s of 0.01 (orange) and 1 (gold) is, again, not dramatically pronounced, suggesting that the changing amount of phage are not influencing the implicated burst time.
Determination of burst time using varied adsorption time and consistent MOI. A new lysate was used for this experiment, and re-titered prior to calculation of MOI. All host cultures were grown and then diluted to an OD of around 0.25 before being infected by Donny at an MOI of 0.01. Each group was performed in triplicate in addition to a control sample, with 1 mL readings taken every ten minutes until depletion of the liquid culture. Adsorption times of 1, 5, 10, and 15 minutes were observed under the assumption that Donny may be infecting host at a higher rate than previously believed, and lysis is still detectable by previously determined methods. Procedures were performed in pairs and on different days, with the 1 min and 15 min adsorption sets sharing a control group, and the 5 min and 10 min adsorption sets sharing a different control group; this staggered approach was done due to time constraints. After analysis of the data, however, the MOI’s of the 1 min and 15 min sets was determined to be 0.015 due to a miscalculation of liquid culture volume and cfu/mL. Results are summarized in Figure 10a-d. The newfound difference in MOI was assumed negligible and the results were graphed accordingly. A one-way ANOVA was performed across all four groups and the controls using SPSS software in order to discern a significant difference between any groups. Differences between total averaged control OD’s (M = .36, SD = .09) and averaged experimental OD’s of all varied adsorption groups (M = .25, SD = .01) suggests that experimental growth was slower overall. Results showed that there was a significant difference between groups in terms of control times, F(3, 50) = 3.90, p < .05, and average experimental times, F(3, 50) = 20.82, p < .001. A Tukey’s post-hoc test showed that the 10 min group (M = .42, SD = .12) had a longer time than other groups, and this difference was significantly significant (p < .05). Further post-hoc analyses showed that, for average experimental times, all groups except the 15 min group were different from one another, and this difference was statistically significant (p < .05 - .001).
Figure 10a. **Optical Density of Donny-infected *M. smegmatis* with 1 minute adsorption.** Both the control and the average of the triplicate data for each time point are depicted. Both cultures were diluted with Middlebrook 7H9 media to an OD around 0.25 before infection at an MOI of ~0.01, and subsequently measured every ten minutes after adsorption. Standard Error of the Mean is represented by error bars on the “1 min average” line. Growth of the infected culture is seen to consistently remain lower than the control sample, with death of multiple cells potentially occurring just at 90 minutes after a mere 1 minute adsorption. OD readings were carried out until all of the culture was depleted.

Figure 10b. **Optical Density of Donny-infected *M. smegmatis* with 5 minute adsorption.** Both the control and the average of the triplicate data for each time point are depicted. Both cultures were diluted with Middlebrook 7H9 media to an OD around 0.25 before infection at an MOI of 0.01, and subsequently measured every ten minutes after adsorption. Standard Error of the Mean is represented by error bars on the “5 min average” line. Growth of the infected culture is also seen to stay consistently lower than the control sample. OD readings were carried out until all of the culture was depleted.
Figure 10c. **Optical Density of Donny-infected *M. smegmatis* with 10 minute adsorption.** Both the control and the average of the triplicate data for each time point are depicted. Both cultures were diluted with Middlebrook 7H9 media to an OD around 0.25 before infection at an MOI of 0.01, and subsequently measured every ten minutes after adsorption. Standard Error of the Mean is represented by error bars on the “10 min average” line. Growth of the infected culture is also seen to stay consistently lower than the control sample, similar to Figure 10b. OD readings were carried out until all of the culture was depleted. OD of the control culture starts slightly higher than 0.25 because of a delayed, staggered beginning as the same control used in Figure 10b. The differences in OD between the control and the average, however, still represent the overall trend that infected samples are decreasing in OD.

Figure 10d. **Optical Density of Donny-infected *M. smegmatis* with 15 minute adsorption.** Both the control and the average of the triplicate data for each time point are depicted. Both cultures were diluted with Middlebrook 7H9 media to an OD around 0.25 before infection at an MOI of ~0.01, and subsequently measured every ten minutes after adsorption. Standard Error of the Mean is represented by error bars on the “15 min average” line. Growth of the infected culture is also seen to stay consistently lower than the control sample, similar to Figure 10a, in that possible death of the host culture may be starting. OD readings were carried out until all of the culture was depleted. OD of the control culture starts slightly higher than 0.25 because of a delayed, staggered beginning as the same control used in Figure 10a.
**Discussion**

Determination of the optimal MOI for these experiments began with the generally inconclusive experiment of looking at three different MOI’s at an OD of around 1.0. Literature surrounding phage experimentation use different ratios based on the particular phage, but a lack of work around Cluster B drove this unanswered question of what works best for Donny. Theoretically, a difference would be seen between the MOI’s of 1 and 10, at least, in that more plaques and/or clearing would be seen sooner in the greater ratio of phage to host; however, no visible plaques or significant difference could be determined. Besides the glaring error of neglecting a control, the plating results were uncertain of a difference between the three MOI’s. Literature-based experimentation was decided for the future due to the project’s time constraints in addition to a lack of usable results. Dedrick et al (2013) used an MOI of 1 for infecting host cultures, so that was chosen as the basis for moving forward to reading OD.

A trend in the data appeared as OD measurements were taken in shorter time frames and within a shorter amount of time overall, suggesting a potential time period between for a single replication cycle. Burst time, potentially indicated by the decrease in OD, may be between 75-120 minutes after a specified adsorption time.

Earlier experiments (Figures 9a-b) showed trends that are overall less consistent with this conclusion, as the cultures continued to increase in OD over long periods of time, whereas later experiments (Figures 9c-e) saw a marked difference between the control samples and infected samples. A procedural difference between these sets may account for the results, since earlier infected samples were not diluted to an OD of around 0.25 but instead were infected when the cultures were taken out of the incubator, interrupting their log phase of growth; this could have potentially accounted for the fact that OD increased since host growth may have overwhelmed and
eventually outnumbered the bacteria that were infected with phage. This may also explain the greater rate of OD increase seen in the lower MOI data in Figure 9a. Less, if any, conclusions can be made about the trend seen in Figure 9b since measurements were taken in such large intervals, an experiment performed to see if a burst and subsequent decrease in OD was seen during late infection. However, in early experiments, a dip in the OD of both infected samples is still seen around 90 minutes which is consistent with later experiments that suggest a burst time inclusive of this.

Phage adsorption kinetics are complex, but the rate of adsorption will generally depend on a number of specific parameters such as host cell growth rates, the adsorption rate constant, phage diffusion rates within the media, phage affinity to the host, and cell growth conditions (Storms et al 2015). Donny was shown to have an interesting trend in adsorption time, where even a short one minute period at room temperature may yield new virions after a latent period of virion assembly. However, this is only speculation since no plating was done to confirm the presence of bacteriophage within the cultures at these times. There are also several caveats to using optical density for indicating the amount of viable host cells and detecting lysed cells in liquid culture, especially as *M. smegmatis* is known for clumpy growth that may have underestimated the indicated amount of cells. Alternatively, an assumption is made that the number of viable cells is represented by OD, when in reality there are likely dead cells suspended within the media that influence absorbance as well. More effort is necessary to create a reliable growth curve relating viable colony counts to OD.

Procedural gaps in this research exist due to time constraints on the part of the researcher. The nature of this project included many troubleshooting efforts for both the host cultures and phage lysate that contributed to a slower overall timeline, but which hopefully provides a guide
for future experimentation. Donny’s characterization is an ongoing effort, and this data will contribute to the sparse literature on Cluster B mycobacteriophage. A one-step growth curve including plaque assays may be the next step in confirming or refuting the data presented, especially the time frames proposed as the start of the lytic cycle. Additionally, further genome characterization will benefit as gene expression during early, middle, and late infection may now be chosen for analyzing Donny’s genes using real time PCR.
References