Undergraduate Journal of Experimental Microbiology and Immunology (UJEMI) Vol. 25:1- 9
Copyright © September 2020, M&I UBC

The rate of T7 phage-mediated lysis of *Escherichia coli* growing in exponential phase is not affected by deletion of *rpoS*

Jingyao Zhu, Cai Lan Jennifer Huang, Kamand Doraki, Bryan Lee

Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada

SUMMARY RpoS is an *Escherichia coli* sigma factor shown to regulate genes in response to various environmental and internal stressors. Previous studies have investigated the role of RpoS in a mechanism known as a cross-protection, in which prior exposure to one stressor leads to increased tolerance to a subsequent stressor. Pre-treatment with subinhibitory levels of antibiotics have been used in several studies in an attempt to elicit a delay in phage-lysis, with varying and contradicting results. We propose that the delayed T7-mediated lysis phenotype may be growth phase dependent as the stress response sigma factor RpoS is known to be expressed in stationary phase. Here, we attempted to perform phage lysis assays on wild-type (WT) and *rpoS* knockout strains of *Escherichia coli* strains growing in stationary and exponential phase, to look for differences in the time to observe bacteriophage-mediated lysis. We did not observe differences in the time of phage-mediated lysis between wild type and *rpoS* knock-out strains of *E. coli* growing in exponential phase. An attempt was made to compare phage-mediated lysis time of *E. coli* grown in exponential and stationary phase, however, technical issues related to normalizing optical density prevented meaningful comparisons. In conclusion, our study characterizes the *E. coli rpoS* knock-out strain JW5437-1 at the nucleotide level, compares the growth curves of a wild type and *rpoS* knock-out strain of *E. coli*, and shows that phage-mediated lysis times are not different between these strains when growing in exponential phase, which suggests RpoS plays no role in this phase.

INTRODUCTION

Escherichia coli, like many bacteria, utilizes diverse mechanisms to regulate gene expression in response to environmental stimuli. RpoS (σS) is an *E. coli* sigma factor responsible for regulating genes during stationary phase and starvation and is upregulated in response to external or internal stress (1). Upon activation, RpoS binds to RNA polymerase (RNAP), controlling its association with specific promoter regions in the genome, and hence the transcription of different genes (1). This mechanism allows the cell to respond to a diverse array of stressor signals through transcription-level regulation of the genome.

Global transcriptomes of bacteria show an extensive number of genes being expressed in response to seemingly unrelated stressors (2). Previous studies have proposed that crosstalk between various stress response mechanisms may be involved in cross-protection, a mechanism in which prior exposure to one stressor leads to increased tolerance to a subsequent stressor (2, 3). Given its role as a global regulator, RpoS has often been selected as a primary target of investigation for its potential role in conferring cross-protection (3, 4). However, the role of RpoS in conferring cross-protection is not well understood.

Bacteriophages are viruses that infect and propagate in bacteria and archaea (5). During the lytic cycle, the phage injects their genetic material into a host bacterium and hijack its cellular machinery for replication (5). The phages multiply within the host until eventually the cell lyses and releases viral progeny. A well-characterized bacteriophage known as T7 belongs to the Podoviridae family of viruses and is one of the seven phage types known to grow lytically on *E. coli* (6). T7 development is dependent on the physiological state of the host, which is characterized by levels and activities of host cellular functions such as growth phase, extracellular stimuli, and host metabolism (7).

Studies have proposed that exponential-phase *E. coli*, when pre-treated with subinhibitory levels of antibiotics, develops resistance to T7 bacteriophage-induced lysis.

Published Online: September 2020

Citation: Jingyao Zhu, Cai Lan Jennifer Huang, Kamand Doraki, Bryan Lee. 2020. The rate of T7 phage-mediated lysis of *Escherichia coli* growing in exponential phase is not affected by deletion of *rpoS.* UJEMI 25:1-9

Editors: Daniela Morales and Julia Huggins, University of British Columbia

Copyright: © 2020 Undergraduate Journal of Experimental Microbiology and Immunology. All Rights Reserved.

Address correspondence to: https://jemi.microbiology.ubc.ca/

Cho et al. treated *E. coli* B23 with sub-lethal concentrations of aminoglycoside and beta-lactam antibiotics prior to T7 phage exposure (8). These cells exhibited delayed lysis compared with cells received no treatment (8). However, when the role of RpoS in cross-protection against bacteriophage infection was investigated, it was shown to be non-essential by Krystal et al. (3). Additionally, another study by Beskrovnaya et al. found that antibiotics did not confer any bacteriophage resistance (4).

This discrepancy in results can potentially be explained by asking whether there is a difference in bacteriophage mediated lysis time at different stages of growth. Previous studies on cross-protection have focused primarily on the expression of RpoS in exponential phase *E. coli* (3, 8-10). However, there are studies that have questioned the role of RpoS in exponential phase cells (11-13). RpoS is shown to be upregulated in the stationary phase (2), which is likely a product of increased stress (i.e. nutrient deficiency and/or waste accumulation), that warrants higher RpoS-dependent gene expression (14). If *rpoS* is only expressed at higher levels in stationary phase cells, exponential phase cells treated with sub-lethal concentrations of antibiotics would exhibit little or no delayed T7 phage-induced lysis. As such, we decided to measure T7 lysis times in *E. coli* growing in exponential phase and stationary phase in order to establish the role (or lack thereof) RpoS has in conferring cross-protection against bacteriophage.

We hypothesized that upregulation of RpoS in the stationary phase *E. coli* will lead to delayed phage-mediated lysis, when compared to exponential phase *E. coli.* Additionally, insufficient levels of RpoS in exponential phase *E. coli* will lead to faster onset of phage-lysis when compared to stationary phase cells. To investigate this, we attempted to assess wildtype and *rpoS* knockout *E. coli* strains in both stationary and exponential phase for differences in onset of bacteriophage-mediated lysis. We expected the stationary WT samples to exhibit delayed onset of phage-lysis compared to the exponential WT samples, and both exponential and stationary *rpoS*-knockout samples. However, we were unable to assess the differences between growth phases due to technical issues related to normalizing optical density. Despite this, we found that phage-mediated lysis times are not different between both strains when growing in exponential phase, which suggests RpoS does not play a role in conferring phage resistance in this phase.

METHODS AND MATERIALS

Bacterial strains and bacteriophage used in this study. Wild type *E. coli* K-12 (BW25113) and *rpoS* knockout mutant (JW5437-1) strains were obtained from the in-house collection at the Department of Microbiology and Immunology at the University of British Columbia. All cells were grown in Luria Bertani (LB) medium at 37ºC on a shaker (200 rpm) for 24 hours prior to use. In this paper, strains BW25113 and JW5437-1 are referred to as wild type (WT) and Δ*rpoS*, respectively. T7 phage stock used for propagation was also obtained from the in-house collection.

T7 bacteriophage propagation. 50 µL of WT overnight culture was added to 5mL LB media and incubated at 37ºC on a shaker (200 rpm) until a desired OD_{600} (0.5-1.0) was measured. 1mL of T7 phage stock solution was then added to the cell culture. The infected cells were incubated in a 37°C shaker (200 rpm) until clearing or reduced turbidity was observed. To induce cell lysis and phage release, 1% w/v chloroform was then added into the infected cell cultures. The cultures were centrifuged for 10 minutes (2750 x G, 4°C). The supernatant was filter sterilized using 0.22µm PES membrane filters and the phage stock was stored at 4 °C.

Double agar overlay plaque assay to determine T7 phage titre. 50µL of the WT overnight culture was added to 5 mL of LB liquid media. The subculture was incubated at 37ºC on a shaker (200 rpm) until log phase (OD_{600} ~ 0.5). 100µL of the overnight culture was then mixed with 100µL of T7 phage of varying dilutions (undiluted, 10^{-2}, 10^{-4}, 10^{-6}, and 10^{-8}). The phage-cell mixtures were incubated for 10 minutes prior to transfer into 3mL 0.75% (w/v) LB top agar. The solution was vortexed and poured onto the underlay 1.5% (w/v) agar LB plate. Three replicates were performed, and plates were incubated at 37 °C

overnight. The plaques were quantified on the following day. T7 bacteriophage titer was determined based on plaque forming units (PFU) per mL.

PCR and agarose gel electrophoresis to confirm rpoS knockout mutant (JW5437-1) strain. Colony Polymerase Chain Reaction (PCR) was performed by selecting a single isolated colony and placed it in the PCR mix. Each PCR mix was prepared with 0.4 μM forward and reverse primers, 1.5 mM $MgCl_2$, 0.2 mM dNTP mix, 1x Taq polymerase (Invitrogen) as well as 1x PCR buffer (Invitrogen). Primers were designed to flank the kanamycin resistance gene (Table 1). Three replicates were performed with WT as our positive control. PCR was performed in the Bio-Rad T100TM Thermal Cycler (Bio-Rad Laboratories, Inc.) programed to first lyse the cells by running at 95℃ for 5 minutes. The Thermal Cycler was then run denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, extension at 68 °C for 30 seconds, and repeat for 50 cycles, followed by a final extension at 68°C for 5 minutes. PCR products were then held in the Thermocycler at 4 °C overnight and purified using PureLink PCR Purification Kit (Invitrogen). Both PCR products and a 100 bp DNA marker were run on a 1% (w/v) agarose gel containing SYBR Safe DNA Gel Stain (Invitrogen) in 0.5x Tris/Borate/EDTA (TBE). Gel was run in 1x TBE buffer at 100V for 60 minutes. Bands were visualized using the Gel Doc UV imager. The PCR products were then sequenced and confirmed via Sanger sequencing (Genewiz company). The results were blasted against the reference genome in the NCBI database.

PCR and agarose gel electrophoresis to confirm T7 phage stock purity. Polymerase Chain Reaction (PCR) performed consisted of 1.036×10^{10}M T7 bacteriophage stock, 0.2 μM forward and reverse primers, 1.5 mM $MgCl_2$, 0.2 mM dNTP mix, 1x Taq polymerase (Invitrogen) as well as 1x Taq polymerase buffer (Invitrogen). T7-specific primers were designed to amplify the *gp10A* gene of T7 phage while T4-specific primers, amplifying the *gp23* gene of T4 phage, was also used as a negative control to check for T4 contamination (Table 1). Three replicates were performed. PCR was performed in the Bio-Rad T100TM Thermal Cycler (Bio-Rad Laboratories, Inc.) programed to run denaturation at 95°C for 30 seconds, annealing at 58°C for 30 seconds, extension at 68°C for 30 seconds, and repeat for 50 cycles, followed by a final extension at 68°C for 5 minutes. PCR products were then held in the Thermocycler at 4°C overnight and purified using PureLink PCR Purification Kit (Invitrogen). Both PCR products and a 100 bp DNA marker were run on a 1% (w/v) agarose gel containing SYBR Safe DNA Gel Stain (Invitrogen) in 0.5x Tris/Borate/EDTA (TBE). Gel was run in 1x TBE buffer at 100V for 60 minutes. Bands were visualized using the Gel Doc UV imager. The PCR products were then sequenced and confirmed via Sanger sequencing (Genewiz company). The results were blasted against the reference genome in the NCBI database.

TABLE. 1 PCR primer sets and expected annealing temperature

Gene	Primer Sequence (5'-3')	Tm (℃)	Annealing Temperature (℃)
rpoS	Forward: TGGTGCGTATGGGCGGTAAT	69.6	71.6
	Reverse: TGCCGCAGCGATAAATCGG	68.5	
T7 *gp10a*	Forward: CGAGGGCTTAGGTACTGC	64.7	68.0
	Reverse: GGTGAGGTGCGGAACTTC	65.9	
T4 *gp23*	Forward: GCCATTACTGGAAGGTGAAGG	65.6	66.6
	Reverse: TTGGGTGGAATGCTTCTTTAG	63.2	

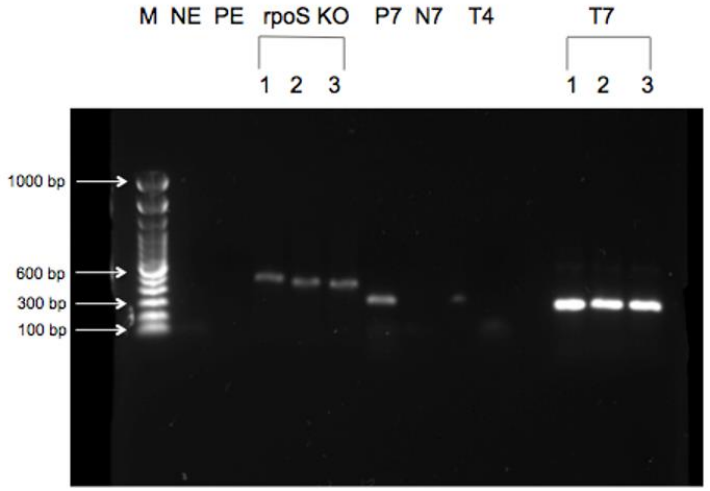

FIG. 1 PCR analysis confirms that the kanamycin resistance cassette was removed from WT to create Δ*rpoS* as well as the presence of T7 *gp10a* gene without T4 phage contamination. PCR was performed using isolated colonies of Δ*rpoS* with *rpoS* primer flanking the kanamycin resistance cassette (*rpoS* KO 1-3). The T7 phage purity was also confirmed with T7 gDNA using both T7 primers (T7 1-3) as well as T4 primers (T4). WT colonies was used as template for our positive control (PE, P7) while distilled water for negative control (NE, N7). Amplicons were resolved on a 1% agarose gel at 100V. Image of the gel was taken using the UV light viewing cabinet. Successful removal of the cassette should yield a

WT and ΔrpoS growth curves. 50 µL of WT and Δ*rpoS E. coli* overnight culture were each added to 5 mL of LB liquid media. The subculture was incubated at 37 ºC on a shaker (200 rpm) till the expected OD_{600} (~0.02). 200 µL of each subture was loaded to the 96well plate. Ten replicates were run with sterile LB as the blank. The plate was incubated at 37ºC in the BioTek Epoch plate reader for 24 hours with linear shaking every minute. Measured OD_{600} was plotted against the time that the samples were taken to obtain the growth curve for both **WT** and Δ*rpoS*. The early exponential phase, late exponential phase and stationary phase were determined based on the curve with a conversion factor of 1 OD_{600}=8×10^8 CFU/mL for cell concentration calculation.

Phage lysis assays. The susceptibility of WT and Δ*rpoS E. coli* strains to T7 phage lysis was first confirmed by infecting a 5 mL overnight subculture sample of each strain with T7 bacteriophage stock solution and observing the clearing of the cell cultures. 50 µL of WT and Δ*rpoS* overnight culture were each added to 5 mL of LB liquid media. The subculture was incubated at 37 ºC on a shaker (200 rpm) until an OD_{600} of 0.1 for exponential phase and 0.6 for stationary phase cells. Bacteriophage T7 was diluted to a multiplicity of infection (MOI) of 0.05 and 0.1, according to the following equation:

$$MOI = \frac{(pfu/mL) \times (mL\ Phage)}{Number\ of\ cells}$$

Ninety µL of WT cells (OD_{600}= 0.1/0.6) were loaded to the 96 well plates, together with 10 µL of T7 phage solutions (MOI=0.05/0.1). Seven replicates were performed with same method repeated for Δ*rpoS*. Sterile LB was used as blank while WT and Δ*rpoS* overnight culture without T7 phage were used as our control. The plate was run in the BioTek Epoch plate reader at 37°C for 2 hours with linear shaking every minute.

RESULTS

PCR analysis and Sanger sequencing confirms the disruption of *rpoS* gene in Δ*rpoS E. coli* strain (JW5437-1). To observe the effects of RpoS during phage-mediated lysis, we compared the lysis curves of the wild type *E. coli* K-12 (BW25113) and Δ*rpoS E. coli* strain (JW5437-1). In order to confirm the *rpoS* knockout in the Δ*rpoS E. coli* strain (JW5437-1), colony PCR and gel electrophoresis were performed. Colony PCR was used to amplify the *rpoS* gene region in the Δ*rpoS E. coli* strain (Δ*rpoS*) and gel electrophoresis was performed to confirm the purity of the colony PCR-amplified products. Colony PCR was successful as the *rpoS* knockout samples all yielded single bands at around 400bp and the negative control did not have any PCR products (Fig. 1). Even though the positive control for the colony PCR failed to work, the positive control for the T7 PCR worked. Since both positive control samples are the same except for the annealing temperature, it indicated that the

positive control in the colony PCR was not successful, possibly due to the low annealing temperature.

In order to observe the knockout in the *rpoS* gene, the PCR-amplified products were then sent to Genewiz for Sanger sequencing. The sequencing result using the *rpoS* forward primer was then aligned against the WT reference genome in the NCBI database. Since JW5437-1 has a kanamycin resistance cassette in place of the *rpoS* gene, successful removal of the cassette should yield a band of approximately 300bp in size, including a start codon, a scar sequence of 81 nucleotides in length, 18 nucleotides in the 3' region of the rpoS gene, and a stop codon (10). The BLAST result showed that the PCR product we obtained from the Δ*rpoS* strain indeed has a missing segment in the middle when compared to the original *rpoS* gene (Fig 2A). The resulting amplicon sequence is 383 nucleotides long with 25 nucleotides aligning with the 5' region and 120 nucleotides aligning with the 3' region of the *rpoS* gene (Fig S1). Within the first 25 nucleotides, we observed a nucleotide sequence that indicates a start codon. Similarly, within the 120 nucleotides in the 3' end there is a stop codon sequence. The middle of the amplicon consisted of a 34-nucleotide-long partial scar sequence called FRT (FLP recognition target) sites (16). This region is seen in the Δ*rpoS* strain indicating that a gene cassette was removed from the strain using FLP recombinase, leaving a FRT sequence (also known as a 'scar sequence') behind (16). Between the partial scar sequence and the regions that match up with the *rpoS* gene, there are sequences that do not match up to either the *rpoS* gene or the scar sequence (19 nucleotides before and 26 nucleotides after the partial scar sequence).

***E. coli* strain JW5437-1 bearing a deletion of rpoS shows decreased growth rate and yield compared to an isogenic wild type strain.** In order to compare the effect of RpoS level in the stationary phase versus the exponential phase during phage-mediated lysis, we need to be able to harvest cells in these particular cell phases. The parameter we chose to use is OD_{600} readings for cells in exponential and stationary phases. To determine the respective OD_{600} readings, we obtained the growth curves for both the Δ*rpoS* and WT

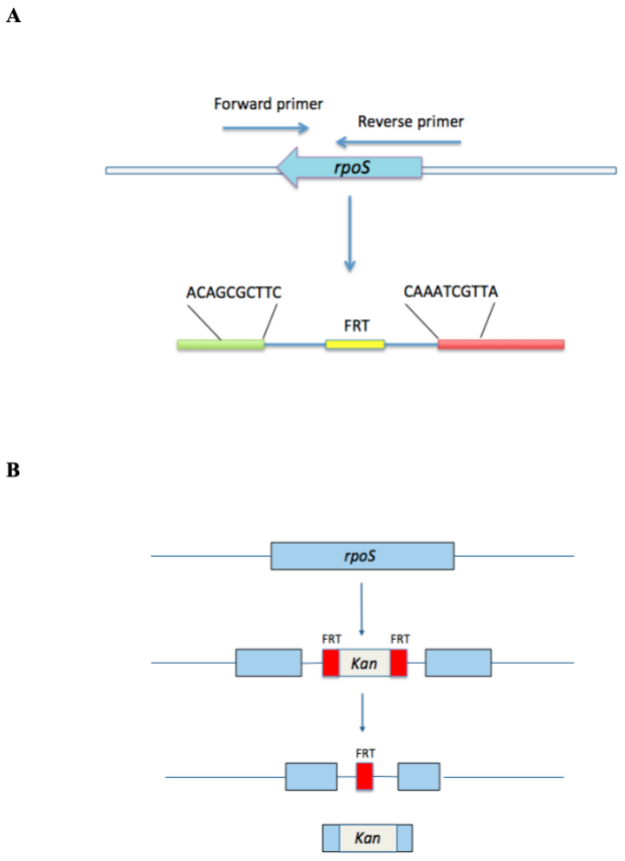

FIG. 2 The *rpoS* gene in the Δ*rpoS* strain is a combination of the front and back portion of the original *rpoS* gene with the kanamycin-resistance cassette (Kan) removed, leaving with a FRT region in between. Colony PCR was performed with *rpoS* primer flanking the kanamycin resistance cassette. The blast result of the PCR product sequence shows that Δ*rpoS* strain has a partially deletion of the *rpoS* gene in the middle replaced by a FRT (FLP recognition target) site. Since the original Kan cassette is flaked with FRT (FLP recognition target) site, the leftover *rpoS* gene sequence in the strain is a combination of the front and back portion of the original *rpoS* gene with a scar sequence in the middle.

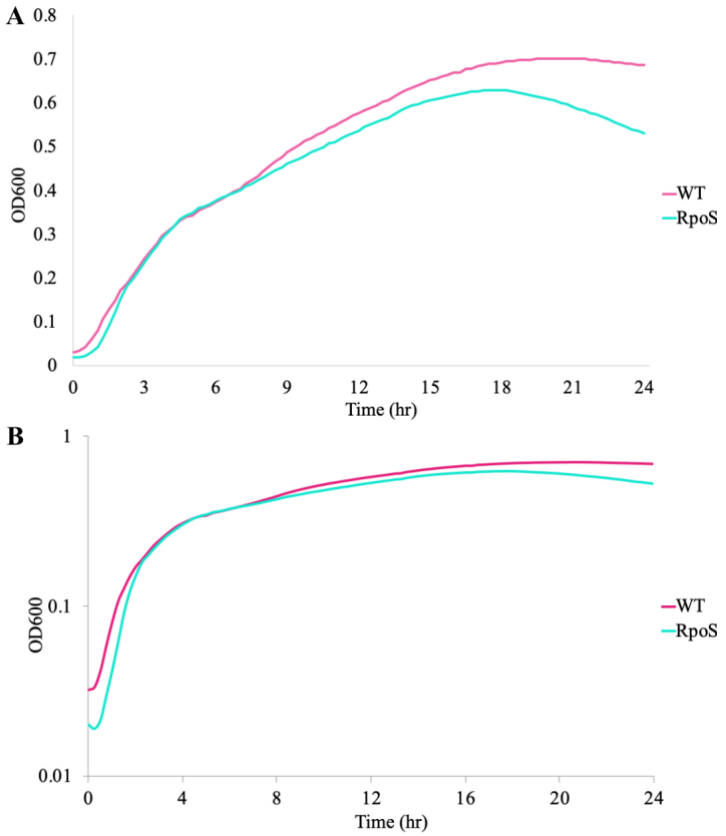

FIG. 3 The Δ*rpoS* and WT strains have distinctive growth curves. On the graph, "RpoS" represents the Δ*rpoS* strain and the "WT" represents the wild type *E. coli* K-12 strain. The growth curves were measured over 24 hours at 37 °C (n = 8) with a constant linear shaking (A). Δ*rpoS* and WT showed similar growth patterns before 6 hours. However, as time progressed, Δ*rpoS* cells grew distinctive slower compared with WT cells. In order to determine the exponential and stationary phases for both strains, a semi-log graph was generated based on the growth curve (B). The exponential phase was determined when OD_{600} readings increased shapely and stationary phase was when the graph plateaued. Hence, an OD_{600} reading of 0.1 and 0.6 were used to harvest cells in exponential and stationary phase respectively.

strains (Fig. 3A and 3B). The OD_{600} readings for exponential and stationary phase were determined to be 0.1 and 0.6, respectively. The Δ*rpoS* and WT had growth curves with distinctive patterns. In the exponential phase, the two strains showed no difference, however, from the start of the stationary phase onwards, the Δ*rpoS* cells started to show a lower OD_{600} readings compared to the WT cells. The difference in OD_{600} readings increased between the two strains as the growth curve proceeded.

Confirming T7 bacteriophage stock solution titering and purity. Based on the double agar overlay plaque assay, the T7 phage stock solution was determined to have a concentration of 2.59×10^{11}T7 phage/mL (Table S1). To determine the purity of the T7 phage stock solution we made, we performed PCR and gel electrophoresis with primer sets designed to amplify T7 *gp10a* and T4 *gp23* genes. The resulting amplified product should be 296bp and 398 bp respectively (3). The gel image showed that there are strong single bands for T7 phage ran with T7-specific primers and no product yield for the sample ran with T4-specific primers (Fig. 1). The strong single bands all have been observed to have a sequence size of around 300bp, which corresponds to the expected size of 296bp. The amplicons were also sent for Sanger sequencing. The sequencing results matched with the bacteriophage T7 genome in the NCBI database (Data not shown). The amplicons confirmed the identity of T7 bacteriophage in the stock solution and the lack of amplicon using T4-specific primers indicated that the T7 stock solution we have is high in purity.

Lower levels of RpoS expression in the WT exponential phase lead to no delayed phage-mediated lysis. In order to test the susceptibility of WT and Δ*rpoS E. coli* strains to T7 phage lysis, we infected an overnight subculture sample of each strain with T7 bacteriophage and looked for the clearing of the cell cultures. Since both samples showed significant clearing compared with no treatments, T7 was confirmed to lyse both Δ*rpoS* and WT strains of *E. coli*. To observe the role of RpoS on delay phage lysis in stationary phase and exponential phase cells, we performed lysis assay on both the WT and Δ*rpoS E. coli* strains using T7 phage. Lysis curves were generated to compare the time of onset for T7 phage-mediated lysis in two different strains to see if there is a delay in lysis. All control samples showed continuous increase in OD_{600} readings indicated that the cells were in a

healthy state and hence the decrease in OD_{600} readings were due to T7 phage lysis in treatment groups. From the lysis curves of the exponential phase cells, it is shown that the Δ*rpoS* strain lysed at the same time as the WT strain. Even though cells in stationary phase showed a delay in lysis in WT compared with Δ*rpoS* strain, cells were started with different OD_{600} readings and hence no conclusion can be drawn confidently (Data not shown).

DISCUSSION

RpoS has been found to regulate gene expression in the stationary phase of *E. coli* growth cycle (1). Studies investigating the involvement of RpoS in decreasing *E. coli* susceptibility to T7 phage lysis found contradicting results (3, 4, 8). When Cho et al. treated *E. coli* B23 with sub-lethal concentrations of antibiotics prior to T7 phage exposure, the cells exhibited delayed lysis compared with cells received no treatment (8). However, when the role of RpoS in *E. coli* was investigated by Krystal et al, it was shown to be non-essential in increasing tolerance against bacteriophage infection. (3). We noticed that previous studies investigating the role of RpoS in relation to *E. coli* phage susceptibility have focused primarily on exponential phase *E. coli* (3, 8-10). Since *RpoS* is a global stress regulator that induces the expression of different stress genes, including genes responsible for phage lysis, its higher expression in the stationary phase compared to exponential phase may be the reason why there are discrepancies between previous studies. (1). As such, we chose to investigate whether or not the presence of RpoS in different phases of growth, specifically between the stationary and exponential phase, has an effect on *E. coli* susceptibility to T7 phage lysis. To further examine the findings of previous research, we hypothesized that the presence of *rpoS* in the *E. coli* genome was involved in delayed phage-induced lysis in the stationary phase, and its absence in the genome or low level of expression in the exponential phase will not lead to a delay in phage induced lysis.

We designed our experiment to compare the effects of different stages of growth on RpoS dependent delay in T7 phage induced lysis. In order to do so, we performed a growth curve analysis to identify the timing and the OD_{600} readings associated with exponential growth phase and stationary growth phase (Fig. 3). We acquired a *rpoS* mutant strain (JW5437-1) to be able to compare it to wildtype (BW25113) as *rpoS* has been proven to be transcribed in stationary phase. We also characterized the *rpoS* mutant using PCR and Sanger sequencing (Fig. 1 and 2). Finally, we designed a lysis assay to measure time of lysis under different conditions (Fig. 4).

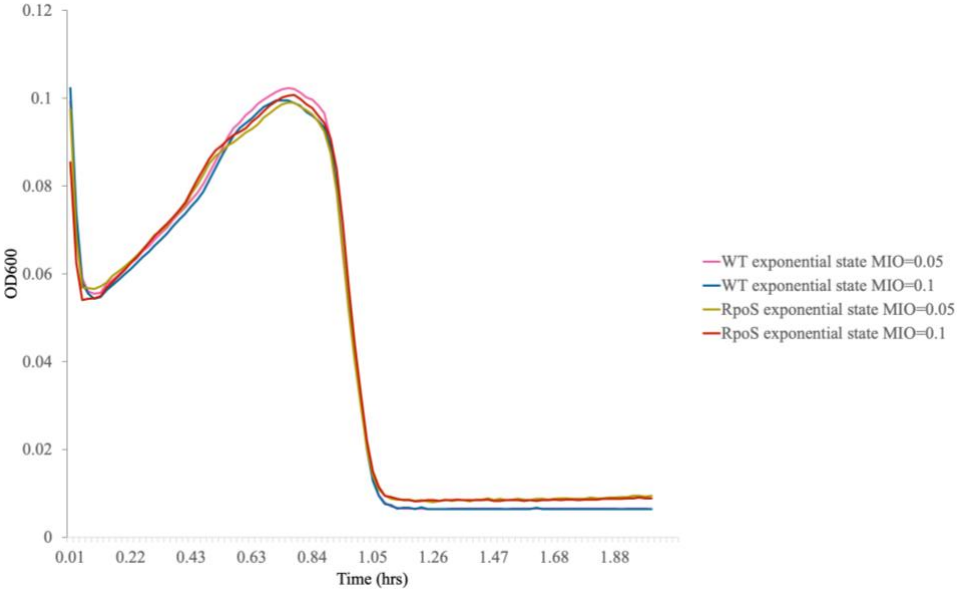

FIG. 4 The WT and Δ*rpoS* cells in exponential phase show no difference in T7 phage lysis time. 90ul of cells in LB and 10uL of T7 phage (MOI = 0.05/0.1) were incubated for 2 hours at 37 °C (n = 7). Both WT and Δ*rpoS* showed similar lysis pattern in exponential phase, indicating the insignificance of *rpoS* in exponential phase cell lysis. Cell lysis was also independent of T7 phage concentration as cells treated with different MOI had consistent lysis pattern.

For this study, we obtained the strains used in a previous study by Krystal et al. The sequencing results show a partial knockout of the *rpoS* gene. This form of gene knockout is likely a result of the removal of the kanamycin-resistance cassette that was initially located within the Δ*rpoS* cell genome. In 2018, Krystal et al. reported that they removed the kanamycin-resistance cassette from the Δ*rpoS* strain (Fig. 2B). Therefore, the leftover *rpoS* gene sequence in the strain is a combination of the front and back portion of the original *rpoS* gene with a scar sequence in the middle.

When comparing the growth curves for WT and Δ*rpoS*, we found that the growth curves showed distinct patterns (Fig. 3). From the growth curves, it was shown that the WT samples were able to grow to a higher final concentration as measured by the higher OD_{600} readings (Fig. 3). This divergence in growth rate between WT and Δ*rpoS* is potentially due to the presence or absence of a functional *rpoS* gene. Δ*rpoS* mutants have been shown to have a lower biomass yield and overall concentration compared to the wildtype (15). Rahman et al. attributed the disruption of growth to the dysregulation in enzyme expression and changes in metabolism due to lack of RpoS activity (15). Hence, the difference in growth curves demonstrate the importance RpoS gene regulation and how it affects the growth rate of *E. coli*. It also indirectly confirms that the Δ*rpoS* mutants we have lack a functional *rpoS* gene, and hence likely have minimal levels RpoS protein expression.

Cells in exponential and stationary phases were collected based on the growth curves OD_{600} readings. T7-phage mediated lysis assay was then performed with different cell phases. The results obtained from the lysis curve analysis showed similar lysis time upon T7 phage infection for both WT and Δ*rpoS* samples in exponential phase. This is in alignment with the findings of Krystal et al., which shows that the loss of RpoS does not result in greater susceptibility to T7 phage-induced lysis in exponential phase *E. coli* (3). From this we can see that RpoS may have little to no role in the mediation of resistance against T7 phage-lysis in the exponential phase. However, for samples in the stationary phase, it is difficult to compare our results due to the discrepancy in the initial OD_{600} values.

Conclusions In this study, we confirmed that the Δ*rpoS* mutant (JW5437-1) has a partial knockout of the *rpoS* gene with a partial scar sequence in the middle. Additionally, the growth curves of the *E. coli* K-12 wild type (BW25113) and Δ*rpoS* mutant (JW5437-1) showed distinctive patterns, which further confirms that the Δ*rpoS* mutant (JW5437-1) lacks a functional growth factor *rpoS*. OD_{600} readings on the growth curves were used to harvest cells in the exponential and stationary phases for the lysis assay. The lysis assay showed that for both *E. coli* K-12 wild type (BW25113) and Δ*rpoS* mutant (JW5437-1) samples in the exponential phase, the time of T7-mediated lysis was similar. For the cell samples in the stationary phase, we were unable to draw a conclusion due to the discrepancy in the initial OD_{600} values.

Future Directions Due to the different starting OD_{600} readings of stationary phase cells, no conclusion can be drawn confidently despite an apparent delay in phage lysis in WT compared with Δ*rpoS*. Hence, in order to confirm the role of RpoS in delay phage lysis, the experiment should be repeated again with same starting OD_{600} readings. Additionally, the RpoS level in different strains should be quantified in order to show a direct correlation between RpoS level and phage-mediated lysis. Hence, for later experiments, western blot analysis should be performed. If our hypothesis is correct, we will see a higher level of RpoS in the WT stationary phase *E. coli* and delayed in phage-mediated lysis when compared to Δ*rpoS* stationary phase cells. Furthermore, since past studies use subinhibitory levels of antibiotic treatment to induce *rpoS* expression while generating distinct results, it would be beneficial for future research to elicit RpoS expression with antibiotic treatment in stationary phase cells to test our hypothesis and also resolve the discrepancy between other studies. Finally, a logical continuation of this experiment could be performing the lysis assay with different bacteriophages, such as T4 phage, to see if there are any similarities to the response seen with T7 phage. This can allow us to know if RpoS plays a role in delayed phage-mediated lysis for T7 phage only or other bacteriophages as well.

ACKNOWLEDGEMENTS

This study was financially supported by the Department of Microbiology and Immunology at the University of British Columbia (Vancouver, Canada). We would like to thank Dr. David Oliver, Dr. Evelyn Sun, Raynold Farrera and Craig Kornak for their support and guidance during this experiment.

REFERENCES

1. **Majdalani N, Hernandez D, Gottesman S.** 2002. Regulation and mode of action of the second small RNA activator of RpoS translation, RprA. *Mol Microbiol* **46**:813–826. doi:10.1046/j.1365 2958.2002.03203.x
2. **Van Bokhorst-van de Veen H, Abee T, Tempelaars M, Bron PA, Kleerebezem M, Marco ML.** 2011. Short- and long-term adaptation to ethanol stress and its cross-protective consequences in Lactobacillus plantarum. *Appl Environ Microbiol*. **77**:5247–5256.
3. **Krystal A, Okamoto R, Sze Andrea, Weiss Z.** 2018. Exposure of Exponential Phase *Escherichia coli* to Ampicillin and Gentamicin Does Not Confer Cross-Protection Against T7 Bacteriophage-Induced Lysis. *JEMI, In Press*. **22**:1-15.
4. **Beskrovnaya P, Janusz N, Omazic L, Perry F.** 2018. Treatment of *Escherichia coli* K-12 with sub-inhibitory concentrations of antimicrobial agents does not induce RpoS mediated cross-protection to T7 bacteriophage infection. *JEMI*. **22**:1-10.
5. **Haq, I.U., Chaudhry, W.N., Akhtar, M.N. *et al.*** 2012. Bacteriophages and their implications on future biotechnology: a review. *Virol J* **9**:9, doi:10.1186/1743-422X-9-9
6. **Drexler H**. 1988. Bacteriophage T1. *The Bacteriophages*. **1**:235-258. doi: 10.1007/978-1-4684-5424-6_7
7. **You L, Suthers PF, Yin J**. 2002. Effects of Escherichia coli Physiology on Growth of Phage T7 In Vivo and In Silico. *Journal of Bacteriology* **184**:1888-1894 doi: 10.1128/JB.184.7.1888-1894.2002
8. **Cho D, Lau I, Li M, Zhu D.** 2017. Development of a Microtiter Plate Assay for Real Times Analysis of T7 Bacteriophage Mediated Lysis of Escherichia coli. *JEMI, In press*. **21**:38-43.
9. **Hardman B, Hunt DJ, Mojaab D, Naor A.** 2017. Sub-Lethal Gentamicin Treatment of *Escherichia coli* UB1005 Induces the Release of Soluble Factors that Reduce Susceptibility to T7 Bacteriophage Infection. *JEMI, In Press*. **21**:128-133.
10. **Huang K, Jang E, Kamma E, Wu A.** 2018. RpoS-Mediated Stress Response Does Not Impact the Rate of T7 Bacteriophage-Induced Lysis of Escherichia coli. *JEMI, In Press*. **4**:1-15.
11. **Wang J-H, Singh R, Benoit M, Keyhan M, Sylvester M, Hsieh M, Thathireddy A, Hsieh Y-J, Matin AC.** 2014. Sigma S-Dependent Antioxidant Defense Protects Stationary-Phase *Escherichia coli* against the Bactericidal Antibiotic Gentamicin. *Antimicrob Agents and Chemother* **58**:5964–5975. doi: 10.1128/AAC.03683-14
12. **Keren I, Wu Y, Inocencio J, Mulcahy LR, Lewis K.** 2013. Killing by Bactericidal Antibiotics Does Not Depend on Reactive Oxygen Species. *Science* **339**:1213–1216. doi:10.1126/science.1232688
13. **Dwyer DJ, Belenky PA, Yang JH, MacDonald IC, Martell JD, Takahashi N, Chan CT, Lobritz MA, Braff D, Schwarz EG.** 2014. Antibiotics induce redox-related physiological alterations as part of their lethality. *Proc Natl Acad Sci USA* **111**: E2109. doi:10.1073/pnas.1401876111.
14. **Vijayakumar SRV, Kirchhof MG, Patten CL, Schellhorn HE.** 2004. RpoS-Regulated Genes of *Escherichia coli* Identified by Random lacZ Fusion Mutagenesis. *J Bacteriol* **186**:8499–8507. doi:10.1128/JB.186.24.8499-8507.2004.
15. **Rahman M, Hasan M, Oba T, Shimizu K.** 2006. Effect of *rpoS* gene knockout on the metabolism of *Escherichia coli* during exponential growth phase and early stationary phase based on gene expressions, enzyme activities and intracellular metabolite concentrations. *Biotechnology and Bioengineering* **94**:585-595
16. **Datsenko KA, Wanner BL.** 2000. One-step inactivation of chromosomal genes in *Escherichia coli* K-12 using PCR products. *Proceedings of the National Academy of Sciences* **97**:6640–6645.
17. **Myers JA, Curtis BS, Curtis WR.** 2013. Improving accuracy of cell and chromophore concentration measurements using optical density. *BMC Biophys* **6**:4.
18. **Bryan, D, El-Shibiny, A, Hobbs, Z, Porter, J, Kutter, EM.** 2016. Bacteriophage T4 Infection of Stationary Phase E. coli: Life after Log from a Phage Perspective. *Frontiers in Microbiology*. **7:**1391. Doi: 10.3389/fmicb.2016.01391. https://www.ncbi.nlm.nih.gov/pubmed/27660625