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# Sub-lethal treatment of *Escherichia coli* strain B23 with either β-lactam or Aminoglycoside Antibiotics May Delay T7 Bacteriophage-mediated Cell Lysis

Haoran Li, Wumian Wang, Yiwei Yang, Myra Lam

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

**SUMMARY** With increasing rates of antibiotic resistance, there is renewed interest in the utilization of bacteriophages to treat pathogenic bacteria. However, the complex interplay between bacteria, phage, and antibiotic treatments is not well understood. A recent study by *Hardman et al.* found that treatment of *E. coli* with sub-lethal concentrations of the aminoglycoside antibiotics gentamycin and kanamycin lead to an increase in T7 phage resistance (7-10). We propose the idea of cross-protection, or a generalized stress response, as a possible framework for explaining and interpreting their results, in which case cells are able to produce a response induced by a primary stressor (sub-MIC antibiotics) and exhibit enhanced tolerance to a secondary stressor (T7 phage infection). We used a real-time cell lysis assay to investigate the effects of sublethal concentrations of antibiotics on phage resistance in *Escherichia coli* B23. We found that both beta-lactam and aminoglycoside increased resistance to lysis by T7 phage. This result suggests that sublethal concentration of antibiotics may decrease the rate of T7 replication and that this phenomenon is not unique to one class of antibiotics. We suggest that delayed T7-mediated lysis of Escherichia coli B23 may be due to upregulation of a general stress response following treatment with sublethal concentrations of antibiotics.

## INTRODUCTION

The rise of antibiotic-resistant bacteria has resulted in an increased interest in bacteriophage therapy. Bacteriophages are ubiquitous viruses that infect and kill bacteria (19). T7 bacteriophage is a species of the *Podoviridae* family, which are tailed phage with a double-stranded linear DNA genome. T7 bacteriophage possess a capsid with diameter of approximately 60 nm, and a 23-nm tail that consists of 6 trimer fibers (1). Attachment and entry of the phage into *Escherichia coli* B23 is mediated by the tail, which recognizes and binds to lipopolysaccharide (LPS) on the outer membrane of the target cell; binding of the tail to LPS, which may also involve porins or outer membrane proteins (2, 3), leads to triggering of conformational changes that inject viral DNA into the cytoplasm (2, 1). The genome is then replicated and transcribed into a polycistronic mRNA that undergoes further processing and translation to yield necessary viral proteins. Approximately five minutes into the infection of the phage, *E. coli* DNA is degraded, and subsequently, phage particle components undergo in vitro packaging to yield viable phage (18). *E. coli* is then lysed via a lysis enzyme, killing the cell and releasing viral progeny (18).

Studies have been conducted to investigate possibility of phage-antibiotic combination therapy (20). Therefore, the interaction between antibiotics and bacteriophage and how the combined effect impacts bacteria is of great interest. The presence of bacteriophage, antibiotics, heat, extreme pH and nutrient deprivation often act as environmental stressors in Gram negative bacteria such as *E. coli.* Stress results in increased expression of RpoS, which is a stress-response regulator that triggers protective changes in cell physiology and metabolic activity (12). A study has shown that bacterial cells, after adapting changes induced by primary stressors, can achieve enhanced tolerance to secondary stressors via a mechanism known as cross-protection (11). Another study has showed general stress response to cold temperature reduced the stability and further reduced the infectivity of bacteriophage lambda, which supports the possibility of stress induced increase in phage resistance via cross-protection mechanism (21). A recent study by *Hardman et al.* reported that treatment of *E.*

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

*coli* with sub-lethal concentrations of the aminoglycoside antibiotics gentamicin and kanamycin as the primary stressor, lead to an increase in resistance when they are incubated with the secondary stressor, T7 phage (7-10). *Hardman et al*. proposed a mechanism in which the release of free LPS that bound to and neutralised the T7 phage receptor, increasing *E. coli* resistance to bacteriophage infection. *Hardman et al* arrived at this model because the mode of growth inhibition of aminoglycosides was known to disrupt cell wall structure and release free LPS (9). However, further data obtained from experimental procedures that involved the addition of free LPS to the antibiotic-treated *E. coli* did showed that there was no decrease in infectivity of T7 (9). It was noticed that the presence of sub-lethal concentration of antibiotics also acted as a stressor which could have triggered general stress response and cross-protection and reduced the infectivity of T7 phage (21).

In this study, the effect of sub-lethal concentration of different classes of antibiotics on T7 phage infectivity was investigated using a 96 well plate assay to measure optical density over time (22). We hypothesize that *E. coli* B23 treated with sub-lethal levels of gentamicin, kanamycin (aminoglycosides) and penicillin and ampicillin (β-lactam) prior to incubation with T7 phage will all lead to a decrease in the infectivity of T7 bacteriophage. In contrast to Hardman *et al*., we focussed on the concept of cross-protection and general stress response; in order to test this concept, we used both aminoglycoside antibiotics and another class, beta lactams. By using different classes of antibiotics that possess different mechanisms of action we will test if reduced in T7 infection is specific to aminoglycosides or if it is a general phenomenon related to stress induced by antibiotic treatment.

## METHODS AND MATERIALS

**Bacterial and Phage Strains**. *E. coli* B23 strain was obtained from the Ramey Strain Collection at the Department of Microbiology and Immunology at UBC. Cultures were propagated in Luria Bertani (LB) broth, with a composition of 1.0% w/v tryptone, 0.5% w/v yeast extract, and 0.5% w/v NaCl, or LB agar plates supplemented with 2.0% w/v agar. T7 bacteriophage was obtained from our in-house stock solution isolated in 2015 (titre of 9.7x10^9 PFU/ml).

**PCR and gel electrophoresis confirmed identity of T7 bacteriophage and phage stock purity**. A polymerase chain reaction and gel electrophoresis assay were done to confirm the identity and purity of the phage stock used in the experimental assays. A fraction of pUC19 and its primers were used as positive control. For the template, a 1/100 dilution of the T7 phage stock was used, and primers specific to the T7 *rpol* gene and the T4 *gp23* gene were used in two separate reactions to confirm the identity of T7 and to check for contamination of T4 bacteriophage. The PCR was run with denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, extension at 68°C for 60 seconds, repeated for 30 cycles, and final extension temperature and hold for 68°C for 5 minutes. For electrophoresis, the PCR products were loaded onto a 2% w/v agarose gel with TBE and SYBR Safe, and run in TBE running buffer for approximately 30 minutes at 70V.

**Minimal Inhibitory Concentration (MIC) Assay**. Minimal inhibitory concentration (MIC) assays were conducted to test each of the following antibiotics: gentamycin, kanamycin, ampicillin, penicillin, azithromycin, and erythromycin. The assays were conducted in 96-well

**TABLE 1 Forward and reverse T7 and T4 primer sequences used to amplify T7 bacteriophage.**

| Description | Fwd. Sequences (5' - 3') | Rev Sequences (5' - 3') | Tm (°C) | % GC Content |
|---|---|---|---|---|
| T7 rpol | CGA GGG CTT AGG TAC TGC | GGT GAG GTG CGG AAC TTC | 52.6 | 61.12 |
| T4 gp23 | GCC ATT ACT GGA AGG TGA AGG | TTG GGT GGA ATG CTT CTT TAG | 54.4 | 52.38 |

plates, with each vertical column representing a serial dilution of the antibiotic of interest. Each horizontal row represented a serial ½ dilution, beginning with 128 µg/ml for the first row, and ending with 0.0625 µg/ml for the last row, for a total of eight rows, which included a positive and negative control. After dilution of the antibiotic concentration, 5µL cell culture prepared from 1/100 dilution of an *E. coli* overnight culture grown to exponential phase (with an approximate OD of 0.3-0.6) was added to each well and incubated for 24 hours at 37°C. The plate was then read at 600 nm using a microplate reader and the OD reading of each well was recorded. The minimal inhibitory concentrations (MIC) were determined by using the highest concentration of antibiotic that had an equal or lesser optical density value compared to the OD of the wells with the negative control. By using the optical density comparable to that of the negative control, we could ensure that cell growth was not inhibited by the antibiotic. The sub-minimal inhibitory concentrations were set as half the MIC (1 ug/mL gentamicin, 2 ug/mL kanamycin, 4 ug/mL penicillin, 0.5 ug/mL ampicillin).

**96-well plate phage infectivity assay.** *E. coli* strain B23 incubated in sub lethal concentration of each antibiotic, were cultured on a shaker in liquid LB media at 37°C overnight. For the purpose of obtaining a synchronously dividing culture in exponential growth phase, a 1/10 dilution was made for each overnight culture with LB supplemented with sub MIC antibiotics. Cultures were incubated on a shaker in liquid LB media at 37°C until an optical density greater than 0.3 was reached (approximately 2 hours). *E. coli* B23 were then diluted to reach a normalized OD600 of 0.3. The amount of T7 bacteriophage required for the desired multiplicity of infection (MOI) was calculated based on the assumption that 1 OD600 corresponds to 8 x 108 cfu/ml. The T7 bacteriophage stock was diluted using LB media. 90 µL *E. coli* B23 culture and 10µL of diluted T7 bacteriophage was added to each well in a 96 well polystyrene plate. A replicate number of 3 was used for each sample. The absorbance at 600 nm in each well was measured on an Epoch Microplate Reader in 10- or 5-minutes intervals. The plate was incubated at 37°C between each read. Positive control was made by adding 10 µL of diluted T7 bacteriophage into 90 µL of the untreated culture in growth phase (in replicates) for each MOI to control for the effects of sub-MIC antibiotic treatment. 100 µL of the antibiotics treated or untreated cultures in growth phase without inoculation of T7 phage served as negative control. 100 µL of LB media were added to single wells and were used as blanks for the plate reader.

## RESULTS

**PCR confirmation of T7 bacteriophage identity.** To verify the identity and purity of our T7 bacteriophage, we performed PCR using a 1/100 dilution of the T7 bacteriophage stock as the template DNA. The PCR was run with primers specific to the T7 *rpol* gene and the T4 *gp23* gene, since both T7 and T4 are studied in our laboratory. Figure 1 shows a band approximately 300bp in length in lane 3 for the PCR run with primers specific to the T7 *rpol*

**Table 2: MIC of selected antibiotics used to treat *E. coli* strain B23. Sub-MIC represents half MIC value.**

| Antibiotic Name | Minimum Concentration (µg/mL) | Sub Minimum Concentration (µg/mL) |
|---|---|---|
| Gentamicin | 2µg/mL | 1µg/mL |
| Kanamycin | 4µg/mL | 2µg/mL |
| Penicillin | 8µg/mL | 4µg/mL |
| Ampicillin | 1µg/mL | 0.5µg/mL |

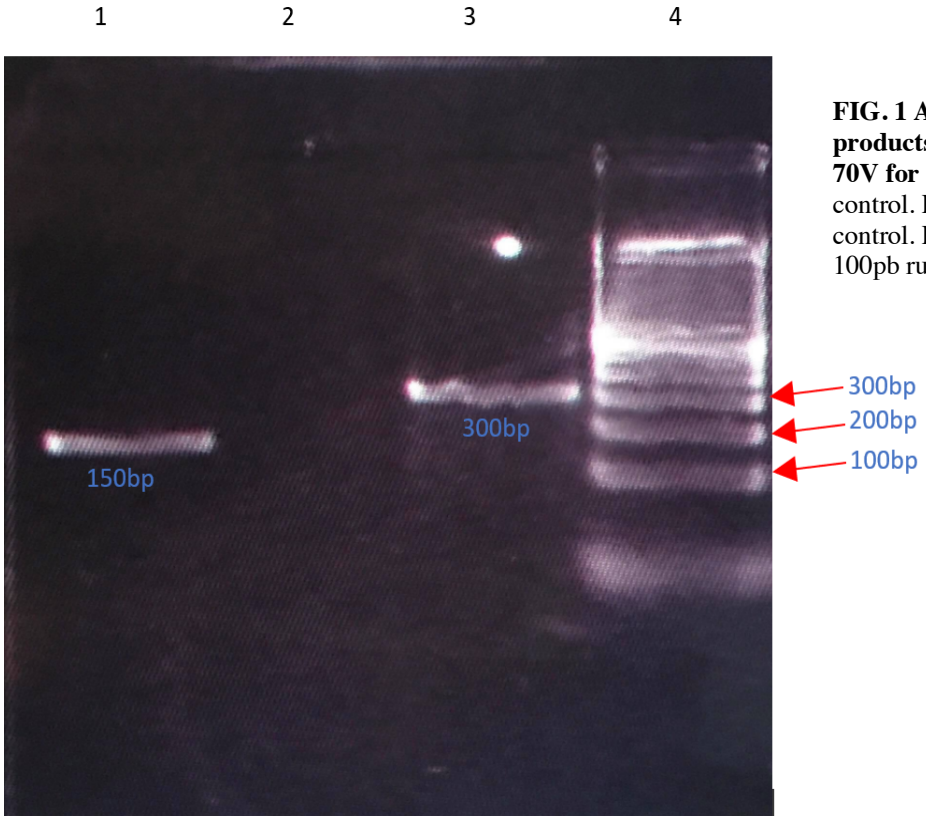


**FIG. 1 Agarose gel (2%) containing PCR products of amplified bacteriophage run at 70V for 30 minutes.** Lane 1, PU19 positive control. Lane 2, T4 bacteriophage negative control. Lane 3, T7 bacteriophage. Lane 4, 100pb running ladder

gene, and an absence of a band in lane 2 for the PCR reaction run with primers specific to the T4 *gp23* gene. The absence of an observable band in lane 2 indicates that no PCR product is observed when amplifying the T7 bacteriophage template with the addition of T4 primers, while the presence of the band in lane 3 shows amplification of the T7 *rpol* gene from the T7 template DNA. Moreover, a band approximately 150bp in length in lane 1 indicates that PCR product of positive control (fragment of pUC19 plasmid) is observed. These data confirm the identity of T7 bacteriophage in our phage stock, and the absence of T4 contamination.

**Minimal inhibitory assay was performed to determine the MIC of various antibiotics, and sub-MIC was established as a two-old dilution of the MIC.** Using a 96-well plate assay, we determined the minimal inhibitory concentrations (MICs) of four different antibiotics on *E. coli* B23: gentamycin, kanamycin, penicillin, and ampicillin. The sub-minimal inhibitory concentrations were determined by using the highest concentration of antibiotic that had an equal or lesser optical density value compared to the OD of the wells with the negative control, which was determined to be approximately 0.05. For gentamycin, kanamycin, penicillin, and ampicillin, the minimal inhibitory concentrations were established to be 2µg/ml, 4µg/ml, 8µg/ml, and 1µg/ml, respectively. We used half the MIC as our sub-minimal inhibitory concentration, the concentration of antibiotic used to pre-treat our *E. coli* B23. At the sub-minimal inhibitory concentration level, all OD readings were above 0.15 and showed signs of normal growth. The results of this MIC assay and the values used for the sub-minimal inhibitory concentration are documented in Table 1. (Supplement Table 1)

**Cells treated with antibiotics at sub-MIC show a delayed and slower decline in optical density in T7 bacteriophage infection.** As shown in Figure 2A, when samples were incubated with T7 phage at MOI=0.1, the untreated sample (blue line) reached peak at 60 minutes and declined to baseline (baseline was OD=0.055 given by blank) by 90 minutes. By comparison, the sample treated with kanamycin (orange line) reached peak at 70 minutes and declined to baseline by 110 minutes. Similar trends were observed for all treated samples Figure 2 (MOI=0.05). Each treated growth condition reached peak OD at a later time and took longer time to reach baseline compared to untreated sample (Figure 2). These data

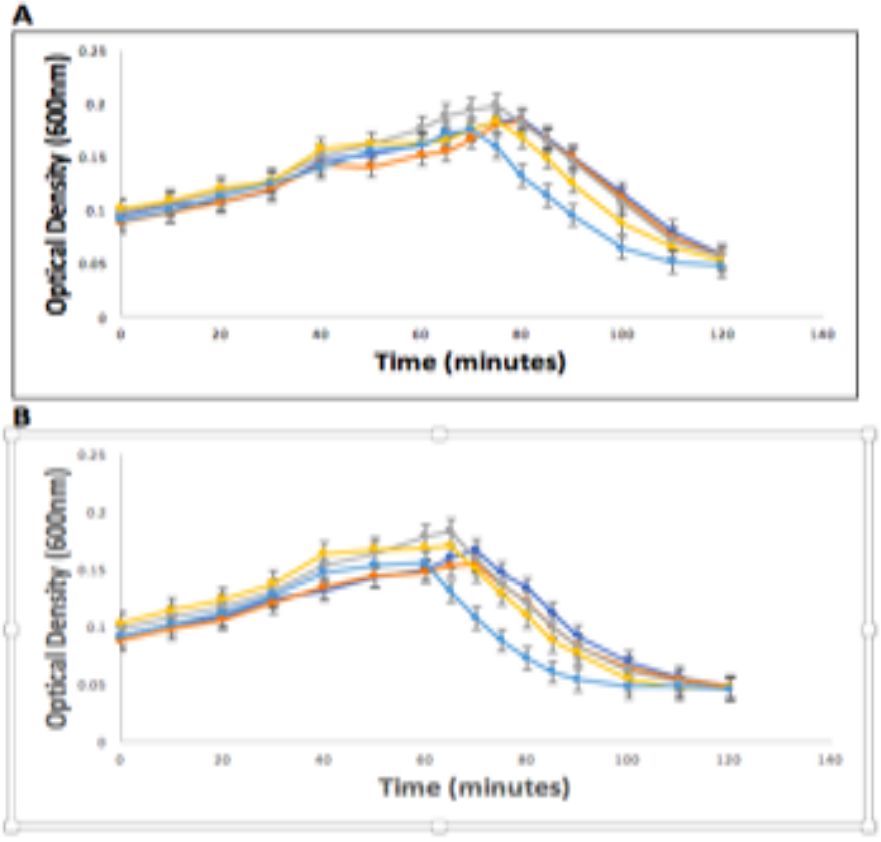


**FIG. 2 Treatment of *E. coli* strain B23 with sub-MIC antibiotics delays T7 induced cell lysis.** Cells were grown in LB broth supplemented with antibiotics to ~ 0.3 OD. Cultures were infected with T7 phage at an MOI of (A) 0.1, or (B) 0.05. Dark blue – untreated, yellow – penicillin, grey – ampicillin, orange – kanamycin, light blue – gentamicin. Standard deviation shown as error bars (n = 3).

indicated that phage induced cell lysis occurred sooner and at a greater rate in untreated sample compared to cultures treated with sub-MIC antibiotics. The data also suggested that phage replication may be slower in cell exposed to both aminoglycosides and β-lactams.Data of the optical density reading at the peak, time point at the peak, time point at the baseline, and time between peak and baseline for each sample are shown in Supplemental Tables 2A and 2B.

## DISCUSSION

Previous studies have used the phage plaque assays to determine the infectivity of T7 phage on gentamycin-treated cells, and determined that sub-lethal levels of antibiotic treatment results in the formation of fewer phage plaques (9, 10). Based on these observations, our research question focussed on the nature of this response. Our goal was to determine whether induced phage resistance was specific to gentamycin, or if it could be induced by antibiotics with different modes of action.

In our approach, we tested the effects of treatment of sublethal levels of antibiotics by measuring the optical density of each well (at 600 nm) at 10-minute intervals over an approximate 1-2-hour period, using an established 96 well plate assay (13). Graphing the OD timepoints produced a cell lysis curve for each antibiotic, which could be used to determine the effect of the antibiotic treatment on cell lysis rates. As previously described by Cho et al., the 96 well plate assay offers an efficient approach to real time analysis of the dynamics of bacteria lysis upon T7 phage infection with less time and labour requirement (13). We found that this assay more efficient, since measuring OD values using a microplate reader presented a much shorter experimental timeframe compared to a phage plaque assay, which requires overnight incubation. In comparison, the time requirement for the establishment of lysis curve from 96 well plate assay is normally 2 to 6 hours depending on the MOI tested. It is also easy to do replicates to increase the statistical robustness of the data. It should be noted that the 96-well plate method is performed with limited agitation (aeration). Evaporation might also occur for experiments running longer than 12 hours.

We performed the 96 well plate assay at an MOI of 0.1 and 0.05. At these MOIs we observed complete lysis curve in approximately 2-3 hours. The cells were grown to exponential phase (~0.3 OD units) which has been shown to be optimal for T7 phage infection (22). We have performed the assay on cells in stationary phase and no difference in peak value reaching time was observed between treated and untreated cells (Supplement Figure1). Narga *et. al* shows that sublethal level of gentamicin would increase *E. coli* B23 resistance to T7 bacteriophage, and they hypothesize this might due to the release of outer membrane vesicles (OMV) (10). Based on previous studies, *E. coli* at this stage would encounter insufficient levels of nutrient (11), and potential response to nutrient depletion is release of outer membrane vesicles (OMV) based on OMV's biological roles (14). Thus, this result is not contradicting to Narga et. al hypothesis (10). T4 bacteriophage have also shown lower infectivity in stationary phase bacteria (15). These data suggest that the ability to observe an effect of sub lethal doses of antibiotics on T7 induced cell lysis may be limited to cell growing in exponential phase.

Employing the 96 well plate assay on exponential phase cells, we demonstrate that the treatment of different classes of antibiotics at sub-MIC confers a protective effect against T7 bacteriophage infection. This is shown by a slightly delayed and slower decline in the lysis curve of treated samples compared to untreated control (Fig. 2A and 2B). At an MOI=0.05, all cell culture conditions were observed to have a delayed OD peak and a longer time needed to reach the lower threshold compared experiments performed on cells at an MOI of 0.1, which is consistent with the idea that fewer phage would result in slower lysis rates. From biological point of view, this indicates that the bacterial response to antibiotics affects resistance against bacteriophage, which may be indicative of cross protection (11). Compared to previous research which assayed plaque production we were able to observed the kinetic effects on lysis after treatment with sub lethal doses of antibiotics (9).

Although a similar trend was observed for all antibiotics, difference in peak values, and the resulting difference between peak and threshold values, were observed among samples treated with different antibiotics. In general, the beta-lactam treated cells showed higher OD peak value than the aminoglycosides. Both antibiotic classes showed a higher peak OD value than the untreated sample. Although it was possible that antibiotic dependent mechanism might have played a role in this difference, the difference could also result from the difference in sub-MIC for each drug, as different OD600 readings were observed for different drugs at selected sub-MIC. The peak time also differed among antibiotics, and beta-lactam showed earlier peak than aminoglycosides at both MOI=0.1 and 0.05. However, since the onset of decline only differed by 5 minutes between two classes, more data should be collected in future experiments at higher resolution (could be achieved by lower MOI) to draw firm conclusions.

A possible mechanism proposed involves the entry of antibiotics into the cells, which disrupts different cellular components and processes depending on the mode of action of the antibiotics. This primary survival stress activates the *RpoS* pathway and upregulates RpoS activity (12), which results in protective changes in cell structure, physiology and metabolic activities and offers cross-protection against secondary stressors (11). Therefore, when the treated cells are inoculated with T7 phage, which acts as a secondary stressor, they exhibit delayed and slowered lysis. This mechanism is proposed based on our experimental findings and background research, and is a possible topic of future interest and research to further elucidate the cellular mechanisms responsible for this response.

**Limitations** The major limitation in our study is that the reproducibility of the experiment is not confirmed by repeating the experiments multiple times. Supplement Fig 2 shows the results obtained from the first 96-well plate cell lysis assay conducted with 6 different antibiotics with no replicates, and the slightly delayed and slower decline was only observed for β-lactams and aminoglycosides but not macrolides. It should also be noted that because the measurements were taken every 10 minutes throughout the experiments, therefore, due to the low resolution and lack of replicates, we could not conclude that sub-MIC treatment of antibiotics delay the T7 phage induced lysis. Figure 2, however, shows the results we obtained from 96-well plate cell lysis assay with modifications, where triplicates were prepared for each sample, and readings were taken every 5 minutes to increase the resolution between 60

and 90 minutes. Although the results shown in Figure 2 should be more reliable, it is worth the efforts to repeat the experiment to verify the reproducibility of the results in the future.

**Conclusions** In conclusion, we found that all treated samples exhibited slightly delayed peak and slower decline in the lysis curve compared to untreated sample, which indicates enhanced resistance to T7 phage infection. These data suggest that antibiotic induced T7 resistance is not unique to gentamicin and may slightly differ between β-lactams and aminoglycosides.

**Future Directions** *E. coli* exposed to beta-lactam can induce RpoS expression, which is a stress-response regulator that triggers protective changes in cell physiology and metabolic activity (12). Whether the activity of *RpoS* is necessary for increasing phage resistant in *E. coli* can be studied by comparing the *E. coli* resistance to T7 phage upon treatment of sub-MIC antibiotics between wildtype and *RpoS* knockout mutant. An increase in T7 phage resistance should be observed in wild-type culture upon treatment while no difference in resistance should be observed in *RpoS* knock out mutant.

In addition to β-lactams and aminoglycosides, we tested effects of macrolides antibiotics including azithromycin and erythromycin (Supplemental Figure 2). The macrolide-treated group shows a flatter lysis curve than cells treated with aminoglycosides or β-lactams. Since the experiment with macrolides was only performed once, we do not know whether the flatter lysis curve is due to unexpected error or is typical for culture treated with sub-MIC of macrolides. Therefore, developing an approach to study the effects of macrolides on T7 induced cell lysis would be a reasonable and insightful approach to furthering investigation on the links between antibiotic-treated *E. coli B23* and T7 phage infectivity.

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## REFERENCES

1. **Bo Hu, William Margolin, Ian J. Molineux, Jun Liu.** 2013. The Bacteriophage T7 Virion Undergoes Extensive Structural Remodeling During Infection. Science. **239:** 576-579.
2. **Vero´ nica A. Gonza´lez-García, Mar Pulido-Cid, Carmela Garcia-Doval, Rebeca Bocanegra, Mark J. van Raaij, Jaime Martín-Benito, Ana Cuervo, and Jose´ L. Carrascosa.** 2015. Conformational Changes Leading to T7 DNA Delivery upon Interaction with the Bacterial Receptor. Journal of Biological Chemistry. **290:** 10038-10044.
3. **Zhao X, Cui Y, Yan Y, Du Z, Tan Y, Yang H, Bi Y, Zhang P, Zhou L, Zhou D, Han Y, Song Y, Wang X, Yang R**. 2013. Outer membrane proteins ail and OmpF of Yersinia pestis are involved in the adsorption of T7-related bacteriophage Yep-phi. J. Virol. **87**:12260-12269.
4. **Hermann T.** 2007. Aminoglycoside antibiotics: old drugs and new therapeutic approaches. Cellular and molecular life sciences, **64**(14): 1841-1852.
5. **Kadurugamuwa JL, Beveridge TJ.** 1997. Natural release of virulence factors in membrane vesicles by Pseudomonas aeruginosa and the effect of aminoglycoside antibiotics on their release. J. Antimicrob. Chemother. **40**:615-621.
6. **Boman HG, Eriksson KG**. 1963. Penicillin induced lysis in Escherichia coli. Microbiology, **31**(3), 339-352.
7. **Chiang S, Koropatnick J, Lam J, Tiu J.** 2010. Salicylate treatment of *Escherichia coli* B23 with capsular polysaccharide induced by sub-lethal levels of kanamycin does not sensitize the cells to T7 bacteriophage adsorption. JEMI. **14**:92-97.
8. **Bleackley J, Cooper J, Kaminski M, Sandilands S.** 2009. The reduction of T7 phage adsorption in *Escherichia coli* B23 cells treated with sub-lethal levels of kanamycin. J Exp Microbiol Immunol. **13**:89-92.
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. **21:**129-133.
10. **Nagra M, Miller R, Francis F.** 2017. Reduced Bacteriophage T7 Infectivity of Gentamicin Treated Escherichia Coli UB1005 Is Likely Not a Result of Treatment-Induced Release of Cellular Factors Into The Culture Supernatant. JEMI. **21**: 46 – 51

11. **Chung HJ, Bang W, Drake MA.** 2006. Stress Response of Escherichia coli. I. Food Tech. **5**: 52-64.
12. **Andersson DI, Hughes D**. 2014. Microbiological effects of sublethal levels of antibiotics. Nau Rev Microbiol. **12**: 465-477
13. **Cho D, Lau I, Li M, Zhu D.** 2017. Development of a Microtiter Plate Assay for Real Time Analysis of T7 Bacteriophage Mediated Lysis of *Escherichia coli.* JEMI, **21**: 38-43.
14. **Kulp A, Kuehn MJ.** 2010. Biological functions and biogenesis of secreted bacterial outer membrane vesicles. Annu. Rev. Microbiol. **64:**163-184.
15. **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. Front Microbiol. **7**:1-12.
16. **Mazzei T, Mini E, Noveffi A, Periti P**. 1993. Chemistry and mode of action of macrolides. J. Antimicro. Chem. **31**: 1-9.
17. **Sailer FC, Meberg BM, Young KD**. 2003. L-Lactam induction of colanic acid gene expression in *Escherichia coli*. FEMS Microbiol Letters. **226**: 245-249.
18. **Kruger DH, Schroeder C.** 1981. Bacteriophage T3 and bacteriophage T7 virus-host cell interactions. Microbiol. Rev. **45**:9-51.
19. **Nilsson AS**. 2014. Phage therapy—constraints and possibilities. U. Jounral of Med. Sci. **119(2)** : 192-198.
20. **Ryan ME, Alkawareek MY, Donnelly RF, Gilmore BF**. 2012. Synergistic phage-antibiotic combinations for the control of Escherichia coli biofilms in vitro. FEMS Immuno & Med Microbiol. **65**: 395-398.
21. **Obuchowski M, Shotland Y, Koby S, Giladi H, Gabig M, Wegrzyn G, Oppenheim AB**.1997. Stability of CII is a key element in the cold stress response of bacteriophage lambda infection. J. Bacterio. **179**: 5987-5991
22. 1. **David Cho, Irene Lau, Michael Li, Dave Zhu Department of Microbiology, Immunology, University of British Columbia.** Development of a Microtiter Plate Assay for Real Time Analysis of T7 Bacteriophage Mediated Lysis of Escherichia coli. **21:**