Journal of Experimental Microbiology and Immunology (JEMI) Vol. 21: 46 – 51

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

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Pre-treatment of *Escherichia coli* UB1005 with sub-lethal concentrations of gentamicin has been shown to reduce the infectivity of Bacteriophage T7. However, levels of infectivity has been shown to increase after a wash following antibiotic treatment. Thus we investigated the cause for the decreased infection of Bacteriophage T7 in *Escherichia Coli* UB1005 after treatment with a sub-lethal concentration of gentamicin. We hypothesized that treatment with sub inhibitory concentrations of gentamicin releases a cellular factor into the supernatant that serves as a decoy receptor to prevent T7 absorption. To test this hypothesis, we transferred supernatants of gentamicin treated UB1005 to untreated UB1005 and tested T7 infection. We observed that the transfer of supernatants from gentamicin treated UB1005 to untreated UB1005 did not reduce the infection of T7 in a double agar overlay assay. These data suggest that reduced T7 infectivity of UB1005 following gentamicin treatment is not due to a released, transferrable, cellular factor in the culture supernatant.

Bacteriophages are viruses that infect bacteria and may cause bacterial cell rupture through lytic replication (1). Due to their antibacterial properties, bacteriophages have been used to treat bacterial infections since the early 1900s, a practice termed phage therapy (2). However, research in phage therapy declined with the discovery of antibiotics which was a more economical method to treat bacterial infections. Today, excessive use of antibiotics has led to selective pressures that favor the evolution and spread of antibiotic resistant bacteria. Phage therapy can provide a practical alternative to treating multi-drug resistant bacterial infections. However, since phage therapy is not well studied, further research is required to understand the factors that might limit its clinical use. One important consideration would be to study the effectiveness of and possible complications arising from simultaneous treatment with antibiotics and phage therapy.

Bacteriophage T7 belongs to the *Podoviridae* family and has a polyhedral head, a simple tail, and one piece of double stranded DNA transcript (1). T7 phage primarily infects *Escherichia coli* (*E. coli)* that has a rough lipopolysaccharide (LPS) rich surface (3). T7 phage undergoes lytic replication and produces progeny virus particles within 12 to 25 minutes at ideal incubation temperatures (3, 4). It is postulated that the viral particle tail adheres to the bacterial cell surface LPS prior to injecting its DNA into the host (3). *In vitro*, studies have shown that the binding of viral particle to bacterial rough LPS is sufficient for the ejection of phage DNA and thus LPS has been recognized as the main receptor mediating phage adsorption and subsequent infection (3).

Pre-treatment of *E. coli* with sub-lethal concentrations of aminoglycoside antibiotics such as kanamycin and gentamicin has been shown to reduce the adsorption and infectivity, respectively, of T7 phage (5, 6). These polycationic antibiotics destabilize bacterial cell walls by substituting the divalent cations between LPS molecules that are critical in maintaining membrane integrity (7). The destabilizing effect on Gram-negative bacteria, including *E. coli*, has been shown to induce the release of blebs of phospholipid bilayer from the outer membrane called outer membrane vesicles (OMVs) (8-10). Consequently, the LPS on the OMV surface may bind T7 phage and reduce levels of phage adsorption as well as infection (10). Garcia-Gonzales *et al.* also suggested that T7 phage binding to the OMV LPS or soluble LPS might lead to ejection of phage genome, essentially inactivating some of the virus (3). However, Hardman *et al* observed that when *E. coli* UB1005 was suspended in minimal (M9) media with solubilized LPS prior to T7 infection, it did not show decreased infectivity (6). This result may have been due to the fact that gentamicin treatment of *E. coli* does not induce a significant release of LPS (11). Furthermore, when gentamicin treated *E. coli* were washed prior to infection with T7, phage infectivity increased compared to that of non-treated bacteria, and suggested a factor present in the culture supernatant that has a protective effect from infection, that when removed increases susceptibility to infection. These studies suggest that an alternative soluble factor in the cell-free media of gentamicin treated cells may be responsible for the observed decrease in T7 infectivity (6).

In this study, we tested whether the reduction in T7 phage infectivity of *E. coli* UB1005 following pre-treatment with sub-lethal levels of gentamicin is due to a soluble, aqueous factor released into the media. We hypothesized that a stress-induced transferrable aqueous factor released in gentamicin treated *E. coli* UB1005 culture was the causative agent for reduced T7 phage infectivity of antibiotic treated bacteria. Contrary to our hypothesis, our data suggests that reduced bacteriophage T7 infectivity of gentamicin treated

E. coli is not a result of treatment-induced release of aqueous factors

MATERIALS AND METHODS

Minimum Inhibitory concentration (MIC) assay. M9 media was used to culture *E. coli* strain UB1005 obtained from Hancock Lab. M9 media is a minimal media used to test stress factors on organisms growing in minimal (12).

Preparation of M9 media. A stock solution of 10X M9 salts were prepared for storage. 60 g Na_2HPO_4, 30 g KH_2PO_4, 5 g NaCl, 10 g NH_4Cl were dissolved in 1000 mL of distilled water. This mixture was sterilized by autoclaving. A 1 M stock solution of $CaCl_2$ was prepared in a separate bottle and sterilize by autoclaving. The M9 media was prepared by mixing together 100 mL of 10X M9 salts, 0.1 mL of 1 M of $CaCl_2$, 50 mL of 10% glucose, 10 mL of 0.1 M $MgSO_4$, 50 mL of 10% casamino acids, 2 mL if 10 mg/ mL vitamin B1, 2 mL of 10 mg/mL tryptophan and 790 mL of deionized water. The mixture was autoclaved and stored on lab bench.

Preparation of gentamicin stock from gentamicin sulphate. 0.5 g of gentamicin sulphate was added to 10 mL of sterile H2O and dissolved by shaking. A 0.22 µm syringe filter was primed by drawing 5 mL of sterile H2O, which was subsequently discarded. The gentamicin solution was filter sterilized using the primed 0.22 µm filter. This stock concentration was stored in -20 0C and used to make further dilutions of working concentrations.

Minimum Inhibitory Concentration Assays. The minimum inhibitory concentration of gentamicin for *E. coli* strain UB1005 grown in minimal M9 media was determined by growing the cells in a 96 well plate Corning™ Costar™ TC-Treated 96 well polypropylene plates (Cat. No. CLS3790) using multiple concentrations of gentamicin. The range of gentamicin concentrations used was from 10 µg/mL to 0 µg/mL. An overnight culture of *E. coli* strain UB1005 grown in M9 media was inoculated at 1:100 dilution and incubated in 37 ºC. The cells were grown until they reached an OD_{600} of 0.4 and 100 µL of this culture was aliquoted into each well of a 96 well plate with a clear bottom. 100 µL of gentamicin solution was added to each well containing cells such that the final concentrations were as expected. Each condition was done in triplicate and incubated over a period of 24 hours at 37 ºC. The plates were visually examined for growth and an OD_{600} reading was taken on the incubated plates using the EPOCH™ Microplate Spectrophotometer by Biotek.

Phage Work:

Viability of the phage. T7 phage stock acquired from the Department of Microbiology and Immunology, UBC was used in this project. The phage was inoculated on a lawn of *E. coli* strain UB1005 grown on 1.5% agar M9 plates using a stab and observed for plaque formations. One of the plaques formed was used to infect a liquid culture of *E. coli* strain UB1005 growing in M9 media using the phage pharming (13).

Liquid Phage Pharming. An overnight culture of *E. coli* strain UB1005 in M9 media was used to inoculate 5 mL of M9 media at a 1:100 dilution. When the culture reached an OD_{600} of 0.5, a sterile toothpick was used to pick one the plaques from the phage viability plate and was dipped into the tube containing the culture. The culture was incubated on a shaker for 7 hours, to provide enough time for the phage to lyse all the cells. 10 drops of chloroform were added to the tube containing the lysate, which was left at 4 ºC over 48 hours. The tube was spun at 5000 rpm at 4 ºC for 12 minutes to separate the chloroform layer containing the cell (13).

Phage purity using PCR. PCR was used to assess T7 phage purity. 1 mL of phage isolate from the aqueous layers was heated on a heating block at 95 ºC. This was done to denature the viral capsid that released the viral DNA. A final total reaction volume of 50 µL comprised of 2 µL of dNTPs, 4 µL of $MgCl_2$, 5 µL of 10X PCR reaction buffer, 1 µL of Forward Primer T7 *rpol* Fwd, 1 µL of Reverse Primer T7 *rpol* Rev, 0.25 µL of Taq Polymerase, 35.75 µL of PCR grade water and 1 µL of template DNA, were set up for PCR. Following a 950 C 10 minute denaturation steps**,** 30 cycles of PCR were run. Each cycle was 5 minutes at 95 ºC (denaturation), 30 seconds at 52 ºC (annealing), 30 seconds at 74 ºC (extension). The reaction was held at 4 ºC once the 30 cycles were completed. The PCR was run on a T100™ Thermal Cycler from Biorad. Two sets of primers were used; one targeting the *rpol* of the T7 phage to check for its presence, and the other targeting the *gp23* of the T4 phage to check for possible contamination of the T7 phage isolate with T4 phage. The sequence (5' to 3' orientation) of the primers used were as follows: **T7 *rpol*:**Fwd: CGAGGGCTTAGGTACTGC, **T7 *rpol*:**Rev: GGTGAGGTGCGGAACTTC, **T4*gp23*:**Fwd: GCCATTACTGGAAGGTGAAGG, and **T4*gp23*:**Rev: TTGGGTGGAATGCTTCTTTAG

The amplicons from the reaction were run on a 1% agarose gel. 0.5 g of agarose was mixed in 50 mL of TAE buffer and heated using a microwave. When the solution was thoroughly mixed, it was allowed to cool to approximately 60 ºC prior to the addition of 4 µL of SYBR Safe. The gel was poured in a cassette and allowed to cool. 9 µL of the PCR reaction was mixed with 1 µl of 10X loading buffer and loaded on the gel. 10 µL of the GeneRuler™ 1 Kb plus was used as a scale for the molecular bands. The gel was run at 110 volts for 35 minutes.

Phage bottom agar and Phage top agar. Phage bottom agar (PBA) was a 1.5% M9 agar that was used for the base. Phage top agar (PTA) was a 0.75% M9 agar that is used for the phage plaque overlay assay. The M9 media was prepared and the appropriate amount of agar was added and autoclaved. The mixture was cooled and poured into sterile petri plates.

Phage titer plaque assay. An overnight culture of the *E. coli* strain UB1005 was set up in 5 mL of M9 media. The culture was seeded at a 1:100 dilution in the required amount of M9 media. It was then grown until it reached an OD_{600} of 0.5. This culture was aliquoted into 1 mL Eppendorf tubes. 0.1 mL of the multiple serial dilutions of the phage were added to these tubes and incubated for 10 minutes at 37 ºC. Aliquots of 3 mL phage top agar were transferred into sterile test tubes and placed in a 47 ºC water bath to prevent solidification. When the 10-minute incubation was complete, the Eppendorf tubes were centrifuged at 5000 x g for 2 minutes and the supernatant was discarded to remove any un-adhered phage. The cell pellet, with the bound phage was suspended with 1mL of fresh M9 media and transferred into 3 mL of PTA. The tube was vortexed to ensure complete mixing and the contents were poured on PBA and swirled to ensure complete spreading. The plates were inverted after the PTA solidified and were incubated at 37 ºC for 6 to 12 hours to allow plaque formation. The plaque forming units (PFUs) were counted for plates that had PFU counts between 20 and 300. The titer was subsequently calculated for the phage preparation.

Sub-lethal gentamicin treatment and T7 infection of *E. coli* UB1005. The effects of gentamicin on the infectivity of T7 phage in *E. coli* strain UB1005 in M9 media was studied by treating aliquots of cells with sub-lethal concentrations of gentamicin prior to infection with T7 phage. *E. coli* UB1005 were grown to an OD_{600} of 0.5 and 1mL was aliquoted into 2 mL tubes. The tubes were centrifuged at 5000 x g for 2 minutes and the supernatant was discarded. The pellets were suspended in 1 mL of M9 media containing gentamicin concentrations of 2.5 µg/mL, 2.0, 1.5, 1.0, 0.5, and 0 µg/mL. 0.1 mL of the 106 dilution of the phage preparation was added and the tubes were incubated at 37 ºC for 10 minutes. The tubes were centrifuged at 5000 x g for 2 minutes and the supernatant was discarded to remove any un-adhered phage.

The pellet containing bound phage was suspended in M9 media with no antibiotic. The cell suspension was added to 3 mL PTA and vortexed to mix. The PTA with the cells and adhered phage were poured on PBA and incubated over 6 to 12 hours. PFUs were counted.

***E. coli* UB1005 supernatant transfer and dilution post incubation in M9 media with gentamicin.** To test whether gentamicin treatment of UB1005 reduces infectivity of T7 phage is due to aqueous factors present in the supernatant we incubated *E. coli* UB1005 for 1 hour at 37 ºC in M9 media containing 2.5 µg/mL of gentamicin. The cells were spun down and the supernatant was transferred into 2 mL Eppendorf tubes containing spun down and decanted *E. coli* strain UB1005 cells that were not treated with gentamicin. The supernatant was diluted with M9 media in a range of dilutions to assess gentamicin-induced effects on the infectivity of the T7 phage. The range of dilutions were all made up to 1 mL, and consisted of six aliquots in duplicates, in which transferred supernatant made up 100%, 80%, 75%, 60%, 50% and 0% of the total 1 mL volume. The individual duplicate dilutions of supernatant were used to suspend fresh cells and 0.1mL of the 10^6 phage dilution was added to each tube. The tubes were incubated at 37 ºC for 10 minutes. The cells were centrifuged at 5000 x g for 2 minutes and the supernatants were discarded to remove any unbound phage. The pellets were suspended in M9 media without gentamicin, mixed with 3 mL PTA, and subsequently plated. The plates were incubated for 6 to 12 hours and the PFUs were counted.

T7 infection post washing of cells incubated in M9 media with gentamicin. To understand the effect of gentamicin on *E. coli* strain UB1005, the cells were incubated in M9 media containing 2.5 µg/mL of gentamicin for 1 hour along with cells with no treatment (control). The cells were spun down and re-suspended in M9 media without gentamicin. 1mL of this suspension was aliquoted into 2 mL Eppendorf tubes. 1 mL of 10^6 dilution of the T7 phage was added to each tube and incubated for 10 minutes. The tubes were centrifuged at 5000 x g for 2 minutes, and the supernatant was discarded to remove any unbound phage. The pellet was re-suspended in 1 mL of M9 media without gentamicin and added to 3 mL of PTA. The tube was vortexed to mix and subsequently plated. The plates were incubated for 6 to 12 hours and the PFUs were counted.

RESULTS

Minimum inhibitory concentration of gentamicin in *E. coli* UB1005. Our goal in this study was to test whether a factor found in the supernatant of pelleted cells caused decrease in T7 phage infectivity following gentamicin treatment of *E. coli*. In order to do this, we needed to pretreat strain UB1005 with sub-minimum inhibitory concentrations (sub-MIC) of gentamicin. To determine the sub-MIC of *E. coli* UA1005 for gentamicin we conducted an MIC assay. Cells were incubated with different concentrations of gentamicin to determine the minimum concentration of antibiotic that inhibits cell growth which represents the MIC (14). *E. coli* incubated in the absence of antibiotic were used as a positive control for growth while sterile LB media served as a negative control. MIC of gentamicin for *E. coli* UA1005 was 2.8 µg/mL. The sub-MIC for each treatment was set at 2.5 µg/mL.

Sub-lethal gentamicin treatment at a MIC of 2.5µg/mL decreases T7 bacteriophage infection in *E. coli* UB1005. Our next step was to repeat the experiment reported by Hardman et al in which increased infection of UB1005 with T7 phage was observed after treatment with sub MIC gentamicin (6). Plaque assays were performed using *E.coli* UB1005 treated with sub-lethal gentamicin concentrations of 0.0 µg/mL, 0.5 µg/mL, 1.5 µg/mL and 2.5 µg/mL. Two uninfected negative controls treated with 2.5 µg/mL and 0 µg/mL of gentamicin were included. Both negative controls showed no plaque formation (data not shown) and sub-lethal gentamicin concentrations of 0 µg/mL, 0.5 µg/mL and 1.5 µg/mL, showed an average of 35.5, 49, and 43.5 plaques, respectively. Treatment with 2.5 µg/mL of gentamicin decreased T7 bacteriophage infection in *E. coli* UB1005 by 1.5 fold compared to the untreated gentamicin control (Fig. 1).

Partial and full supernatant transfer of sub-lethal gentamicin treated *E. coli* UB1005 does not show a decrease in T7 bacteriophage infectivity.

To determine if a factor is present in the supernatant contained protective factors blocking T7 bacteriophage infection, we decided to test if these factors could function in the absence of treated cells. Two batches of *E. coli* UB1005 treated with 2.5 µg/mL of gentamicin or untreated were incubated for 1 hour at 37 ºC. After incubation, they were pelleted by centrifugation and transferred to the untreated decanted samples. The samples were subsequently infected with T7 bacteriophage and were incubated for 10 minutes at 37º C. A plaque assay was performed on these samples. Two negative controls lacking T7 bacteriophage containing either 0% supernatant transfer or 100% were included. Both negative controls showed no plaque formation (data not shown). No significant changes in infectivity were observed from *E. coli* UB1005 cells re-suspended in 0%, 50%, 60%, 75% and 100% treated supernatant (Fig. 2). The data suggest that no protective factor was transferred in this experiment.

DISCUSSION

We studied if pre-treating *E. coli* UB1005 with sub-lethal levels of gentamicin resulted in the release of a protective factor that could be found in the supernatant of pelleted cells, and thus be transferred to untreated cells to confer protection against T7 bacteriophage adsorption. Prior to

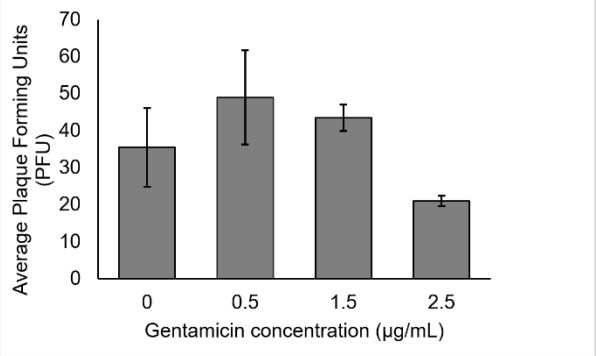

FIG. 1 Sub-lethal gentamicin treatment at a threshold of 2.5µg/mL decreases T7 bacteriophage infection in *E. coli* UB1005. Each bar represents the average PFUs of *E. coli* plated in duplicate. Each duplicate was incubated for one hour in the respective concentration of gentamicin. The error bars represent the standard deviation of the mean.

this study, sub-lethal treatment of *E. coli* with aminoglycoside antibiotics, including gentamicin, were shown to reduce T7 phage infectivity (6, 15). Also, removal of supernatant from gentamicin treated cells followed by a wash step showed increased T7 phage infectivity in *E. coli* UB1005. (6). In addition, previous studies have shown that stressing cells can induce the release of outer membrane vesicles which can act as receptor decoys surrounding the cell, resulting in a positive correlation with survival in the presence of infective phage (9, 10). In some cases, OMVs released by some gram-negative organisms, have been shown to possess degrative enzymes such as proteases and peptidases, which can be speculated to damage phage (8, 16). OMVs may also employ a method resembling expulsion of misfolded proteins from bacterial periplasm to remove toxic and injurious compounds (9). Using this, OMVs could play a role in the removal of antibiotics, and simultaneously, can be speculated to remove any associated phage. These OMV assisted mechanisms may provide a model for antibiotic-induced reduction of phage infectivity in gram-negative organisms. These observations led us to our research question: does the production of protective factors by *E. coli* UB1005 after treatment with gentamicin inhibit T7 bacteriophage infectivity?

In this paper, we show that treatment of *E. coli* UB1005 at a minimum inhibitory concentration of 2.5 µg/mL of gentamicin, exhibited a decrease in T7 bacteriophage infectivity by a factor greater than 1.5 when compared to the control (Fig. 1). This agrees with the findings of Hardman *et al.* where a similar decrease in infectivity (2 fold) was shown (6).

Figure 2 shows that transferred supernatant from gentamicin treated *E. coli* did not decrease T7 bacteriophage infectivity. Cells re-suspended in 'M9 media only' showed similar plaque counts as cells re-suspended in 'M9 supplemented with gentamicin culture supernatant'. This finding supported our null hypothesis; that treatment with gentamicin does not induce the release of a protective factor found in the supernatant of pelleted cells. We were unable to determine the centrifugation rate of particles in M9 media that remained suspended in the supernatant. With this knowledge, we would be better able to identify molecules that fell within that range, and narrow down on the possible factors that either could or could not confer protection. For example, density gradient centrifugation is often used to separate intracellular organelles by their density (17). Once their isopycnic position is reached within the gradient, the organelles will float and can then be compared to coloured beads with known densities (17). This method could be used to determine if OMVs, or other factors released by cells, are present in the supernatant and are not pelleted along with the cells. It is possible that the protective factor was pelleted with the treated cells and was never transferred. Some lysed cell debris may pellet with the cells to which the T7 bacteriophage could bind irreversibly. This cell debris could also include other receptors that the phage interacts with and that may play a role in adsorption (e.g. Omp channel porin proteins) that are suggested to have an effect on viral infection kinetics (3). Moreover, cell debris could also act as a physical barrier to T7 phage adsorption throughout the duration of incubation. Most aminoglycoside antibiotics, including gentamicin, are bactericidal and increasing concentrations of gentamicin could lead to increased amounts of cell debris (18). This would explain why the decreased infectivity was shown with treatment of 2.5 ug/mL gentamicin, but could not be transferred since the hypothetical causative factor was possibly pelleted with intact cells during the centrifugation steps in the supernatant transfer experiment.

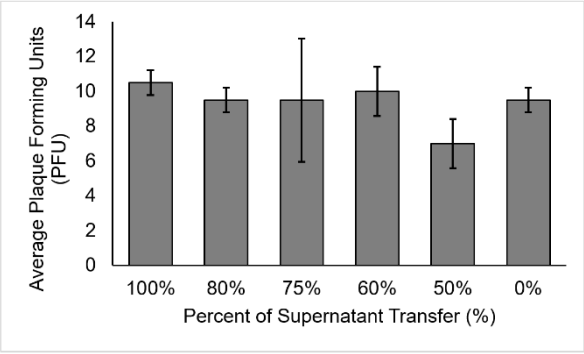

FIG. 2 Partial and full supernatant transfer of sub-lethal gentamicin treated *E. coli* UB1005 does not show a decrease in T7 bacteriophage infectivity. Each bar represents a respective percent of supernatant transferred to *E. coli* prior to a ten-minute incubation with T7 bacteriophage. Biological replicates were plated in duplicate and the average was calculated. The error bars represent the standard deviation of the mean. Gentamicin was added at a concentration of 2.5µg/mL to the samples from which the supernatant was taken.

An attempt was made to repeat the experiment reported by Hardman *et al.* in which the supernatant of gentamicin treated UB1005 *E. coli* was demonstrated to have protective factors acting against T7 bacteriophage infection (6). In this experiment, cells were treated with sub-lethal concentrations of gentamicin for 1 hour at 37 ºC. They were then pelleted and the supernatant was washed away and replaced with fresh M9 media prior to infecting with T7 bacteriophage. This was then compared to an untreated sample. Hardman *et al.* show increased infectivity in cells treated, washed and infected in this manner. When we attempted to repeat this assay, we observed too few plaques to draw a conclusion, but all our plates showed similar plaque counts (data not shown). Due to limiting amounts of data in this experiment we were not able to draw firm conclusions from our data.

Interestingly, the MIC of 8.0 µg/mL determined by Hardman *et al.* was notably higher than 2.8 µg/mL

measured in this study (6). This may have contributed to our lack of positive results in testing the effect of supernatant of gentamicin treated cells on the inhibition of T7 phage infection. Effects could have possibly been observed if a higher concentration of gentamicin was used, since the removal of supernatant showed an increased infectivity in *Hardman et al*'s experiment, using a gentamicin concentration of 8.0 µg/mL. However, this MIC difference could be attributed to several factors, including the stock storage conditions and differences in the method used to determine MIC such as observing growth on a 96 well plate using a spectrophotometer, compared to visually detecting growth in a test tubes of different concentrations of gentamicin, the latter of which was used by Hardman *et al*. This difference may arise due to the higher specificity of the 96 well plate reader due to spectrophotometrically reading turbidity at OD_{600}, whereas visually observing differences in growth rate is less sensitive. Confirming the turbidity with 96 well plate and using smaller increments as used in this experiment can give a more specific MIC as well. The quality of gentamicin used might have affected the quantification of MICs. Gentamicin used by us was freshly stocked and prepared and may have been a more potent source of pure gentamicin. Gentamicin is known to degrade under liquid storage conditions in the presence of light, thus new stock concentrations should be made for every MIC assay to obtain a true representative concentration (19).

We did not investigate if decreased infectivity could be related to changes in the outer surface of *E. coli* UB1005. Liu *et al*. show a correlation between increased capsule size and kanamycin resistance from *E. coli* B23 pre-treated with sub-lethal levels of kanamycin (20). The decreased infectivity could be a result of capsule biosynthesis producing a potentially cell-associated factor that reduces infectivity of the phage, and would agree with our results. This, however, would not explain the result that Hardman *et al.* observed, where the removal of supernatant after sub-lethal gentamicin treatment, and suspension in fresh media prior to T7 infection shows an increase in infectivity of T7 phage (6).

We repeated an experiment performed by Hardman *et al.* and obtained similar results, showing sub-lethal treatment of *E. coli* UB1005 decreases T7 bacteriophage infectivity. The proposed aqueous factor in the supernatant might not confer protection against T7 bacteriophage infection. Hence, we suggest widening the scope for exploring other possible resistance conferring factors. Other factors that may have played a role in the results observed here include lysed cell debris acting as a decoy for irreversible phage adherence, or the physical blocking of the phage due to non-specific binding. Another possibility is that up-regulation of capsule production may have blocked T7 bacteriophage adherence, however, neither one of these postulates have been tested.

Sub-lethal gentamicin treatment of *E. coli* UB1005, reduces the infectivity of bacteriophage T7. This is observed to occur at a minimum inhibitory concentration of 2.5 µg/mL gentamicin, which is lower than the observed MIC of 2.8 µg/mL. This effect was not titratable by transferring multiple dilutions of the supernatant of cells treated with gentamicin, to cells not incubated in gentamicin. Thus, we concluded that the observed effect of reduced infectivity is not due to a gentamicin treatment-induced factors that are transferrable using the supernatant of incubated cells.

FUTURE DIRECTIONS

The inhibitory effect of the sub-lethal levels of gentamicin concentration were not found to be titratable. We conducted an experiment where the cells incubated in gentamicin were washed, and subsequently infected with T7 phage; This experiment yielded inconclusive results due to too low of a phage titre used. This might have been due to minor differences in the execution of the two experiments at different points in time. It would be valuable to combine the supernatant transfer and gentamicin wash experiment, to get more representative and accurate results. The cells incubated in gentamicin, from which the supernatant is removed for the supernatant dilution experiment can be used to wash and be infected with T7 phage. This would reduce the number of variables introduced compared to conducting these experiments separately. The incubated cells that were washed could provide an insight into the possibility of the outer membrane modifications on *E. coli* strain UB1005; or factors that are pelleted along with cells during incubation with gentamicin, which affect T7 phage adherence and infectivity; whereas the supernatant could provide insight into possible factors released during gentamicin treatment of the cells that might interfere with phage adherence and infectivity. Since the cells treated with gentamicin are tested for membrane changes and the supernatant of the incubation is tested for factors inhibiting phage infectivity, the two results are comparable with higher confidence as subtle changes in the experimental procedures such as density of cells used can be eliminated.

Over the course of this project, the temperature of the water bath used to keep the PTA from solidifying was changed constantly to optimize the plaque assay. A temperature study could be done to observe the effects of changes in temperature of PTA between 45ºC and 55ºC, on the representation of the plaques of a given dilution of phage. This will provide valuable information regarding the temperature dependent effects of the plaque formation on phage overlay assay. To observe how the changes in temperature might be affecting the results, we can differentially heat the cells and the T7 phage separately and infect with non-heat-treated phage and infect non-heat-treated cells respectively in a 96-well plate format. The effects can be observed using a spectrophotometer reading

of the 96-well plate to quantify and map the relative changes in the growth profiles. This can also help compare the phage assay to another platform for quantification of the T7 infectivity of *E. coli* strain UB1005.

Other strains of bacteria that are susceptible to T7 phage can be used to compare the effect of gentamicin on T7 phage infectivity. Gentamicin resistant and other strains with high LPS production or outer membrane vesicle production can be used to see if these changes affect T7 phage infectivity while keeping phage titre and experimental conditions constant. The effects of media on the T7 phage infectivity can be explored by repeating this assay in other media types and conditions.

The differences in the infectivity of T7 phage for *E. coli* strain UB1005 at different stages of the growth curve can also be done to observe and alleviate any possible differences in T7 phage infectivity introduced by the growth phase that the cells are in. To explore the possibilities of the effects of gentamicin on T7 phage, the treatment of T7 phage with gentamicin prior to infection should be considered.

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