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O16 Antigen Confers Resistance to Bacteriophage T4 and T7 But Does Not Reduce T4/T7 Adsorption in *Escherichia coli* K-12.

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SUMMARY Expression of O16 antigen in MG1655 *Escherichia coli* K-12 was previously shown to be sufficient to confer resistance to T4 bacteriophage mediated cell-lysis. It was further demonstrated that O16 antigen confers resistance against T4 bacteriophage by preventing adsorption. The main purpose of this research project is to test whether these findings can be replicated with a different bacteriophage, T7. Two *Escherichia coli* K-12 substrains were used in this experiment; MG1655, which does not produce O antigen, and its isogenic strain, DFB1655 L9, which produces O16 antigen via rescue of the *wbbL* gene. These strains were isolated and identities confirmed via colony PCR. T4 and T7 phage lysates were generated and the identities confirmed by PCR. Susceptibility to T4/T7 infection was tested by growth curve analysis and double agar overlay plaque assay. An adsorption assay with qPCR analysis was performed to test for differential bacteriophage adsorption between MG1655 and DFB1655 L9. Our results indicate that DFB1655 L9 is resistant to both bacteriophages, suggesting that O16 antigen is sufficient to confer resistance against T4 and T7 mediated cell lysis. However, contrary to previous findings, we did not observe any difference in adsorption between MG1655 and DFB1655 L9 for either T4 or T7.

INTRODUCTION

O antigen is part of the lipopolysaccharide (LPS) found on the outer membrane of Gram-negative bacteria (1). It consists of oligosaccharide repeating units (O units) made up of two to eight residues from a broad range of sugars (1). O antigen structure varies with sugar subunit, arrangement, and linkages. (1). Most *E. coli* K-12 strains lack O antigen and are described as 'rough', whereas the presence of O antigen is described as 'smooth' (2). Some O antigens serve as attachment points for bacteriophage, while others have been shown to increase resistance to some bacteriophages (2).

Wild-type *E. coli* K-12 does not synthesize O antigen due to mutations in the *rfb* gene cluster, which codes for genes involved in O antigen production (2). One gene located within this cluster is *wbbL*, which codes for rhamnose transferase required for O antigen synthesis (2). *E. coli* K-12 substrain MG1655 has an inactivated *wbbL* gene due to the presence of a 1,195 base-pair IS5 insertion element (2). The restoration of a working *wbbL* gene in MG1655 successfully restored O16 antigen synthesis, and the corresponding substrain was termed DFB1655 L9 (2).

T-even bacteriophage, including T4 and T7, are lytic, double-stranded DNA viruses that infect Gram-negative bacteria, including many strains of *E. coli* (3). In order for a T-even bacteriophage to infect a susceptible cell it must successfully transfer its viral genome into the cytoplasm. The first, stage of viral infection is adsorption, which refers to the binding of the phage tail fibers to receptors on the surface of the cell (3). Binding allows for the formation of a trans-envelope channel that connects the virion tail tip to the cell cytoplasm, through which the viral genome is then transferred (3). The core region of LPS on *E. coli* K-12 strain has been identified as the primary receptor for bacteriophages T4 and T7 (4). In addition to LPS, both phages are thought to interact first with a surface molecule that allows correct tail orientation relative to the bacterial envelope - OmpC in the case of T4, and OmpA/OmpR in the case of T7 (7,10).

Chiu *et al.* demonstrated that the expression of O antigen synthesis in *E. coli* K-12 strain MG1655 is sufficient to reduce bacterial lysis upon exposure to T4 bacteriophage (5). Expanding on this work, Wachtel *et al.* demonstrated that the presence of O16 antigen results in up to a 20-fold reduction in virus detection by qPCR in culture supernatants following

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incubation with E. coli expressing O antigen (6). A steric hindrance model, in which O16 antigen sterically hinders T4 phage from binding to the core region of LPS, thereby preventing adsorption, was suggested as a potential explanation (6). This model put forth by Wachtel *et al* suggests a non-specific mechanism to confer resistance to bacteriophage infection, implying that O16 could confer resistance to a range of different bacteriophage.

Bacteriophage T7 primarily infects 'rough' *E. coli* K-12 strains and has been shown to utilize LPS as a binding point for adsorption (7). Given this, we hypothesize that O antigen expression will result in increased detection of T7 in supernatants following exposure to *E. coli* suggesting reduced adsorption. We predict that O16 will sterically hinder T7 from accessing its binding site on LPS, thereby preventing adsorption. To investigate whether O16 antigen confers resistance to T7, a growth curve analysis and double agar overlay plaque assay was performed. An adsorption assay coupled to qPCR analysis was performed to test whether O16 antigen blocks adsorption of T7. Our results indicate that O16 antigen does indeed confer resistance to both T4 and T7 infection. However, we report that the magnitude of resistance conferred by O16 antigen does not agree with the previous findings from Wachtel et. al.

METHODS AND MATERIALS

***E. coli* K-12 strains used in this study.** Substrains MG1655 and DFB1655 L9 have been previously used in our laboratory, however, they were originally a gift from Dr. Douglas F. Browning from the Henderson laboratory at the University of Birmingham (6). MG1655 is commonly used in the laboratory and does not express O-antigen due to an IS5 insertion within the *wbbL* gene of the *rfb* locus (6). DFB1655 L9 was created by rescuing O16 antigen synthesis in MG1655 by introducing a functional *wbbL* gene (6).

Isolation and identification of *E. coli* K-12 substrains MG1655 and DFB1655 L9. The following methods were adapted from Wachtel *et al* (6). MG1655 was grown on 1.5% agar LB plates. DFB1655 L9 was grown on 1.5% agar LB plates supplemented with 50 µg/mL kanamycin. Both strains were then incubated overnight at 37°C. These plates were used as working stocks of each *E. coli* substrain throughout the project. Colony PCR of the *wbbL* gene was performed on both MG1655 and DFB1655 L9 to confirm strain identities. The expected PCR product size was 799bp for DFB1655 L9 and 1994bp for MG1655. The primers used in this experiment were designed by Browning *et al.* and were used previously in the lab by Wachtel *et al* (2). The PCR mix was prepared using Platinum Taq DNA Polymerase kit, according to the manufacturer's instructions, primers were used at final concentrations of 0.2 µM per reaction. A micropipette tip was used to scrape part of an isolated colony and resuspended in the PCR mixture. The PCR reaction was then performed in a Bio-Rad T100™ Thermal Cycler, with the following conditions: 5-minute initial denaturation at 95°C, 95°C denaturation phase for 30 seconds, 55°C annealing phase for 45 seconds, 75°C extension phase for 2.5 minutes, repeat the mentioned steps for 30 cycles. The PCR products were visualized on a 0.8% agarose gel using 1x TAE buffer at 90V for 80 minutes, stained with SYBR Safe DNA Gel Stain under UV. The primers, which were designed by Wachtel *et al.,* are found in Table 1 (2).

Table 1. Primers used for Colony PCR to amplify *wbbL* gene to confirm *E. coli* K-12 substrains MG1655 and DFB1655 L9, and PCR primers used to Confirm bacteriophage identities to amplify gp23 in T4 and gp10a in T7.

Gene	Sequence (5'-3')	Size (bp)
wbbL	**F:** CCCGAATTCATATGGTATATATAATAATCGTTTCCC **R:** CCCAAGCTTCTCGAGTTACGGGTGAAAAACTGATGAAATTC	1994 (MG1655) 799 (DFB1655 L9)
Gp23 (T4)	**F: GCCATTACTGGAAGGTGAAGG** **R: TTGGGTGGAATGCTTCTTTAG**	398
Gp10a (T7)	**F: CGAGGGCTTAGGTACTGC** **R: GGTGAGGTGCGGAACTTC**	295

Bacteriophage T4 and T7 lysate propagation and identification. The following methods were adapted from Wachtel *et al* (6). In order to generate a working stock of T4 and T7 phage lysates, an overnight culture of MG1655 in LB was prepared. The overnight culture was diluted by 1/5 in 5 mL of LB, before inoculating the dilution with 10uL of T4 and T7 bacteriophage in the respective lysates. The lysates were incubated overnight at 37°C on a shaking platform (200 rpm) to generate lysate. The following day, 300 µL of chloroform was added to the lysate, vortexed and left overnight at 4°C to settle. Finally, sterile filtration was performed on the settled lysate to generate a working stock. To confirm the phage identity and purity, PCR analysis of T4 and T7 working stocks was done. Primers were designed by Browning *et al*. and used previously by Wachtel *et al* (2). The identities of the bacteriophage were confirmed by specifically looking for a 398 bp product representing gp23 for T4, and a 295 bp product representing gp10a for T7. The PCR was conducted using the Platinum Taq DNA Polymerase kit, with the final concentrations of the primers at 0.2 uM per reaction.

1µL of phage lysate was added to each reaction sample and the PCR was performed in a Bio-Rad T100™ Thermal Cycler with the following conditions: 2-minute initial denaturation at 95°C, 95°C denaturation phase for 45 seconds, 51°C annealing phase for 30 seconds, 75°C extension phase for 30 seconds, and repeat these steps for 30 cycles. Finally, the PCR products were visualized on 1.2% agarose gel using 1x TAE buffer at 110V for 50 minutes, stained with SYBR™ Safe under UV. The primers were described by Wachtel *et al* (2).

Assessment of differential susceptibility to T7 or T4 bacteriophage infection of MG1655 and DFB1655 L9. The following methods were adapted from Chiu *et al* (5). Overnight cultures of MG1655 and DFB1655 L9 were prepared in 5 mL of LB. The following day, the overnight cultures were diluted by 1/50 to a total volume of 50 mL in LB supplemented with 1mM $CaCl_2$. Flasks were incubated at 37°C, 200 rpm until reaching an OD600 of at least 0.1. 50 µL of T4 or T7 was added to treatment flasks, with none added to the uninfected controls. Cultures were then incubated at 37°C, 200 rpm, and the OD_{600nm}- was measured at 30-minute intervals using Pharmacia Biotech Ultrospec 3000

Double agar overlay assay to assess differential susceptibility to phage infection between MG1655 and DFB1655 L9 and to enumerate T4 and T7 phage titer. The following methods were adapted from Wachtel *et al.* (6). Overnight cultures of MG1655 and DFB1655 L9 were prepared in 5mL of LB. LB supplemented with 1mM $CaCl_2$ was prepared for the underlay agar using an agar density of 15g/L. The LB supplemented with $CaCl_2$ was prepared for the overlay agar using an agar density of 4 g/L. After autoclaving, approximately 18-25 mL of the underlay agar solution was poured into plastic petri dishes, and the overlay agar

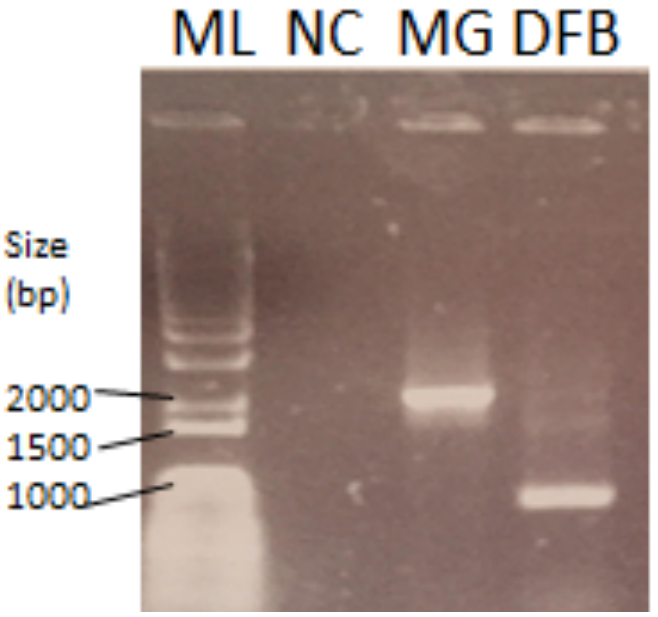

FIG. 1 PCR of *wbb*L gene in *Escherichia coli* strains MG1655 and DFB1655 L9. Samples run on 0.8% agarose gel. The expected PCR products are observed, indicating a non-functional 1994bp *wbbL* gene for MG1655 and an intact 799bp *wbbL* gene for DFB1655 L9. The lane labelling corresponds to the following: ML = molecular ladder, NC = negative control, MG = MG1655 PCR product, DFB = DFB1655 L9 PCR product.

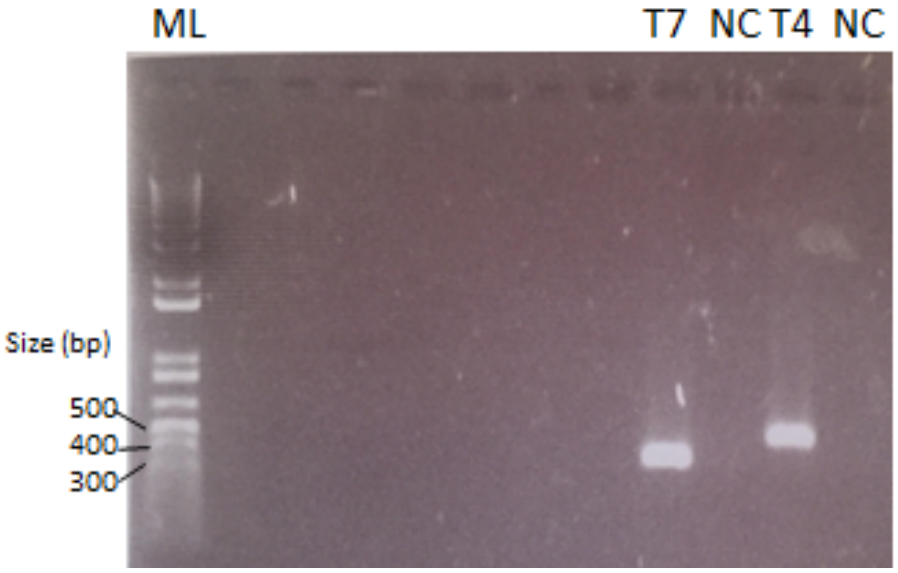

FIG. 2 PCR of gp10a and gp23 in T7 and T4, respectively. Samples run on 1.2% agarose gel. The expected PCR products are observed; a 295bp PCR product indicative of gp10a amplification for the T7 DNA, and a 398bp PCR product representing gp23 for the T4 DNA. The lane labelling corresponds to the following: ML = molecular ladder, T7 = T7 stock phage DNA, NC = negative control, T4 = T4 stock phage DNA

was distributed into 3mL aliquots. Both were cooled and stored at 4ºC. Prior to use, all glass tubes carrying overlay agar broth were passed over a Bunsen burner to melt the solution and then placed in a 55ºC water bath to prevent solidification. The underlay agar plates were placed into the 37ºC incubator for 1 hour to dry any remaining condensation prior to plating. Serial dilutions were prepared of the previously purified T4/T7 lysate (10^{-1} to 10^{-9}) in LB. 100µL of the lysate dilutions were mixed with 100µL of 1.7×10^{9} MG1655 or DFB1655 L9 cells in the liquid overlay agar and plated. Plates were incubated overnight at 37ºC and the number of plaque-forming units (PFUs) were counted the following day.

T4/T7 adsorption assay and qPCR quantification of the supernatant. The following methods were adapted from Wachtel *et al.* (6). T4 and T7 phage was incubated with each *E. coli* strain at equal volumes and decreasing multiplicities of infection (MOIs) from 10^{-3} to 10^{-8}. Staggering the timing, 100uL of a diluted phage was added to 900uL of an *E. coli* cell suspension. The next phage dilution was added every 4 minutes and incubated on the lab bench for 5 minutes. This short incubation time allows bacteriophage to adsorb but does not induce cell lysis (6). After incubation, the cells were centrifuged for 3 minutes at 16,000 x g and 800uL of the supernatant was immediately transferred to a new sterile centrifuge tube. 100uL chloroform was added to disrupt any remaining cells and prevent viral replication, and the samples were stored in a 4ºC fridge. These steps were performed for both T4 and T7 phage. qPCR reactions of the supernatants were prepared in the biosafety cabinet, using Thermofisher Power SYBR Green Master mix according to manufacturer's instructions. Primers were used at final concentrations of 0.1µM. The PCR reaction was performed in a Bio-Rad T100™ Thermal Cycler with the following conditions: 10-minute initial denaturation at 95°C, 95°C denaturation phase for 15 seconds, 50°C annealing phase for 30 seconds, and repeat these steps for 39 cycles. Finally, a melting curve analysis was performed. Data was collected in Bio-Rad CFX Manager™ Software and analyzed in Microsoft Excel.

RESULTS

PCR analysis of the *wbbL* gene for strain identification yields the expected size PCR products for *E. coli* MG1655 and *E. coli* DFB1655 L9. In order to confirm the identities of the strains used in the experiment, PCR amplification of the *wbbL* gene was performed and the bands were compared to their expected sizes. Gel electrophoresis of our PCR samples loaded onto a 0.8% agarose gel was performed, followed by UV gel imaging to visualize the bands. MG1655 has an expected *wbbL* band size of 1994bp, which is indicative of a non-functional *wbbL* gene. DFB1655 L9 has an expected *wbbL* band size of 799bp, which is indicative of an intact, functional *wbbL* gene. As seen in Figure 1, both strains have the expected product size corresponding to the non-functional *wbbL* gene in MG1655 and the

functional *wbbL* gene in DFB1655 L9. No unexpected PCR products were found in either sample or in the negative control. These results indicate that the isolated MG1655 and DFB1655 L9 have the expected genotype.

PCR analysis of the bacteriophage lysates yields the expected size PCR products for bacteriophage T4 and T7. As both T4 and T7 will be used in the project, it was necessary to confirm the identity of each of the working stocks of bacteriophage. Bacteriophage lysates were prepared for each bacteriophage and PCR amplification was performed using separate sets of primers for capsid proteins gp23 in T4 and gp10a in T7. The expected PCR product size for T4 is 398bp, indicative of gp23 amplification. The expected PCR product size for T7 is 295bp, indicative of gp10a amplification. PCR products were run on a 1.2% agarose gel and visualized with UV imaging. As seen in Figure 2, the PCR products for T4 and T7 are consistent with the expected size. No unexpected PCR products were found in either sample or in the negative controls. These results indicate that the working stocks of T4 and T7 are correct and contamination with either T4 or T7 was undetected using PCR.

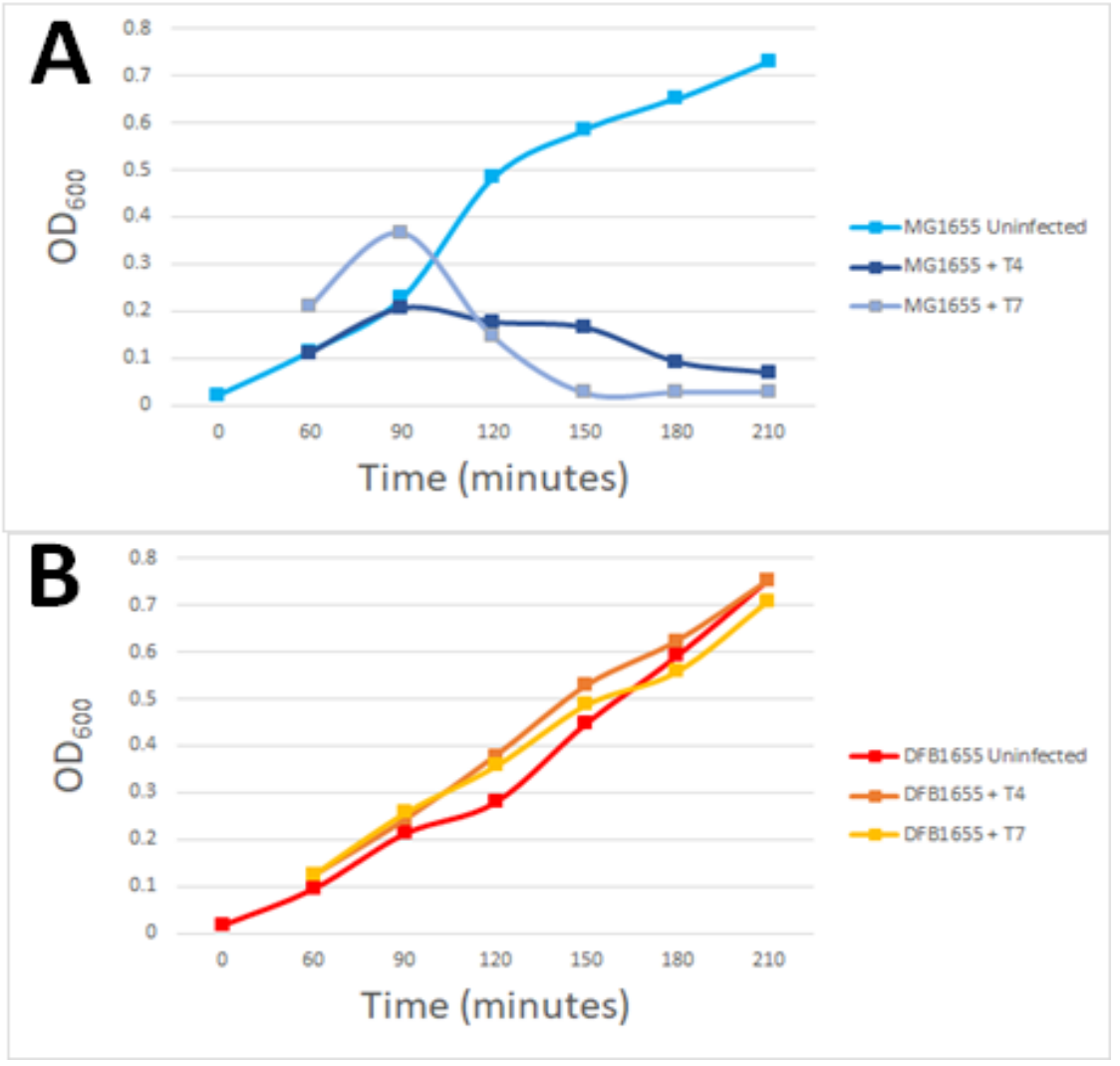

FIG. 3 Growth curve analysis shows differential susceptibility to T4 and T7 cell mediated lysis between MG1655 and DFB1655. A) Growth curve of MG1655 inoculated at time zero with either T4 or T7 bacteriophage (MOI = 0.21). MG1655 uninfected control displays normal, uninterrupted growth. MG1655 infected with T4 and T7 both display reduced growth by 120 minutes, and an OD_{600} near zero by 210 minutes. **B)** Growth curve of DFB1655 L9 inoculated at time zero with either T4 or T7 bacteriophage. DFB1655 L9 displays the same, uninterrupted growth across all three conditions.

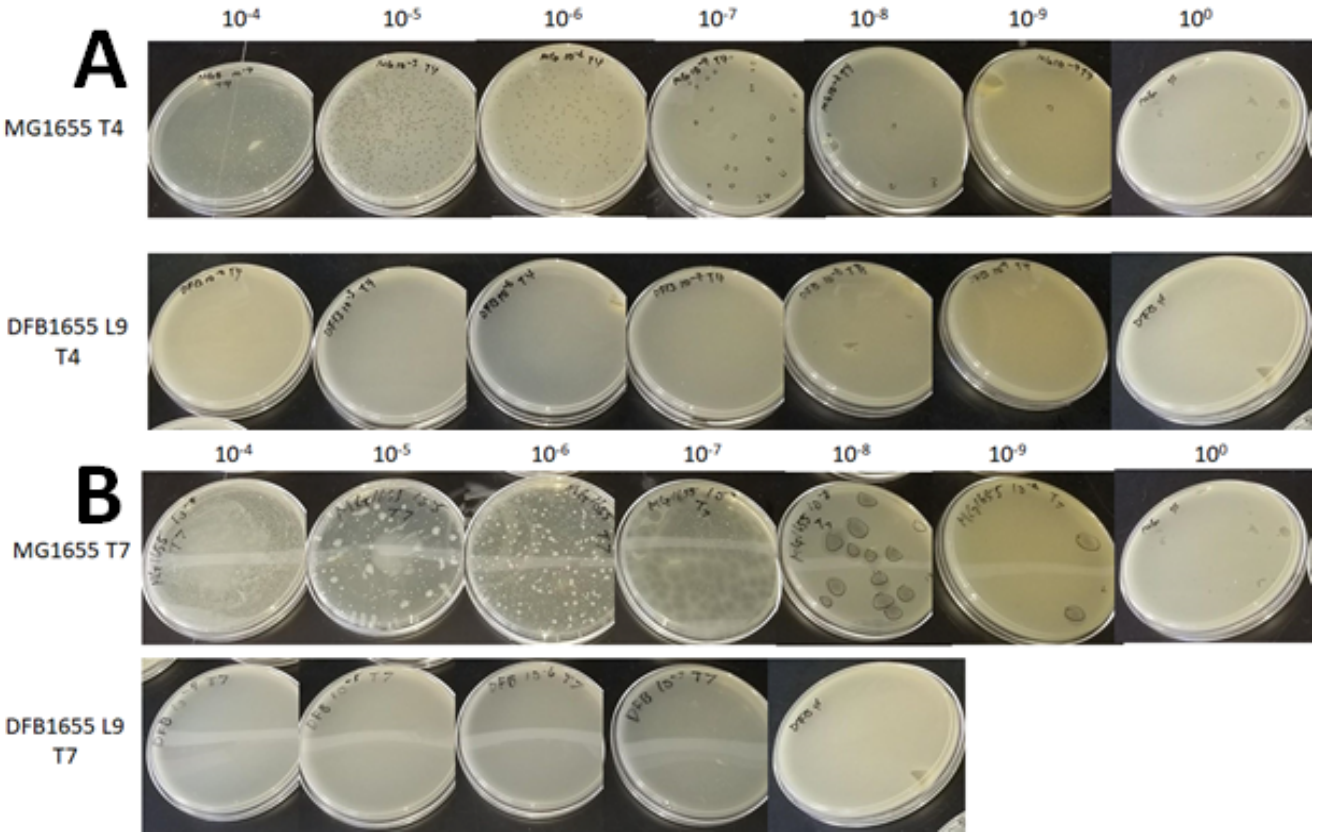

FIG. 4 Double agar overlay plaque assay showing differential susceptibility to T4 and T7 mediated cell lysis between MG1655 and DFB1655 L9. A) Serial dilutions of T4 phage incubated with either MG1655 or DFB1655 L9 **B)** Serial dilutions of T7 phage were plated on LB plates containing $CaCl_2$ with either MG1655 or DFB1655 L9 and incubated at 37°C overnight.

DFB1655 L9 displays resistance to bacteriophage T4 and T7 mediated cell lysis. In order to test for susceptibility to T4 and T7, we measured *E. coli* growth following inoculation with bacteriophage. A growth curve of each strain, MG1655 and DFB1655 L9, was generated following inoculation with either bacteriophage T4 or T7. Figures 3A and 3B show the growth curve generated using optical density measurements of MG1655 and DFB1655 L9, respectively. The uninfected MG1655 and DFB1655 L9 controls both exhibit exponential growth. MG1655 infected with T4 and T7 both show a decrease in optical density by 120 minutes post inoculation and reach an optical density near zero by 210 minutes post inoculation. This is indicative of T4 or T7 bacteriophage mediated cell lysis. DFB1655 L9 infected with T4 or T7 both exhibit exponential growth similar to the uninfected sample. These results indicate that the presence of a function *wbbL* gene, and therefore expression of O16 antigen, is sufficient to confer resistance to T4 or T7 mediated cell lysis.

A double agar overlay plaque assay was performed in order to further test for differential susceptibility to T4 and T7 between strains. Serial dilutions of T4 phage or T7 phage were plated on LB plates containing $CaCl_2$ with either MG1655 or DFB1655 and incubated at 37°C overnight. Susceptibility to bacteriophage is measured by the number of plaques formed on the plate. Figures 4A and 4B show plaques formed following infection of MG1655 and DFB1655, respectively. In these figures, MG1655 inoculated with T4 and T7 shows increasing numbers of plaques as phage concentration increases, suggesting susceptibility to T4 and T7. DFB1655 L9 inoculated with T4 and T7 does not result in any plaques, suggesting that DFB1655 L9 is not susceptible to T4 or T7. Phage titers were calculated to be 2.4×10^{9} PFU/mL for MG1655 and 1.7×10^{10} PFU/mL for DFB1655 L9. These results further indicate the presence of functional *wbbL* gene, and therefore the expression of O16 antigen, is sufficient to confer resistance to T4 and T7 mediated cell lysis.

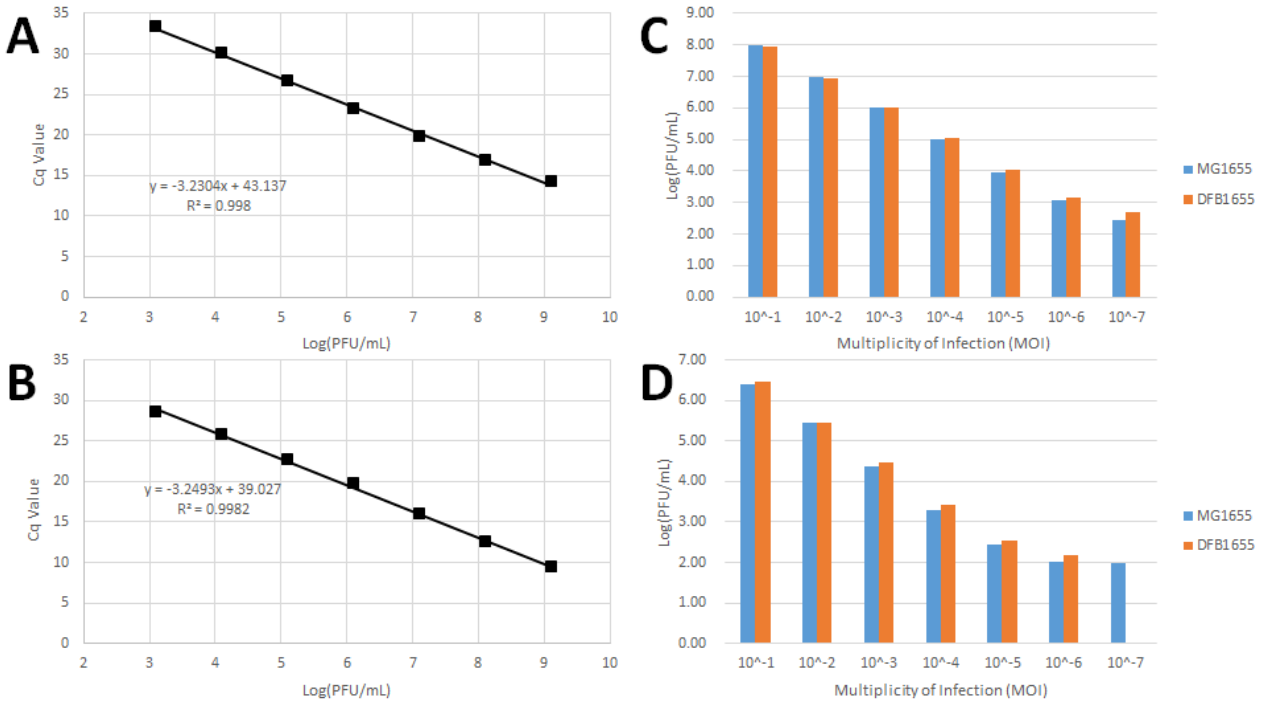

FIG. 5 qPCR quantification of T4 and T7 assay. qPCR of supernatants of MG1655 and DFB1655 incubated with T7 or T4. **A)** Standard curve of T4 bacteriophage qPCR generated via amplification of gp23. **B)** Standard curve of T7 bacteriophage qPCR generated via amplification of gp10a. **C)** Enumeration of T4 left in the supernatant for varying multiplicities of infection (from 10^{-1} to 10^{-7}). **D)** Enumeration of T7 left in the supernatant for varying multiplicities of infection (from 10^{-1} to 10^{-7}), representing the concentration of T7 which was not adsorbed.

No observed difference in T4/T7 bacteriophage adsorption between MG1655 and DFB1655 L9. In order to test if there are differences in T4 or T7 adsorption between MG1655 and DFB1655 L9, a bacteriophage adsorption assay was performed in which each bacteriophage was incubated with each *E. coli* strain at equal volumes and decreasing multiplicities of infection (MOI) from 10^{-3} to 10^{-8}. The sample was left to incubate for 5 minutes, giving phage time to complete adsorption into the cells but not to replicate in the cells. The supernatant was collected, and treated with chloroform to lyse remaining cells and prevent virus replication. Phage detected in the supernatant did not adsorb to the cell. qPCR was run on the collected supernatants to quantify T4 or T7, as well as on T4 and T7 standards of known quantities. T4 and T7 phage in the supernatant was quantified by comparing the qPCR Cq value to the generated standard curve. Figures 5C and 5D compare T4 and T7 infection, respectively. At each MOI for both T4 and T7, there is less than 2 Cq difference between the two bacterial strains. When converted to phage concentration via standard curve, the results show less than 1 PFU/mL difference in adsorption of T4 or T7 in MG1655 and DFB1655. If O16 antigen blocked T4 or T7 adsorption, the quantity of phage found in the supernatant of DFB1655 L9 would be expected to be higher than in MG1655.

DISCUSSION

O16 antigen dependent resistance to T4 lysis for E. coli in liquid culture was demonstrated by Chiu et. al., who showed identical growth curves of DFB1655 with or without T4 infection. (cite) Wachtel *et al.* demonstrated that the presence of O16 antigen results in up to a 20-fold reduction in virus detection by qPCR in culture supernatants following incubation with E. coli expressing O antigen (6). The model for O16 antigen conferring resistance to T4 and T7 was thought to be due to steric hindrance, in which O16 antigen physically prevents access of the LPS core domain by the phage tail domain (6). Expanding on this previous work, we hypothesized that O16 antigen would also confer resistance to T7 bacteriophage and would result in decreased adsorption of T7, as T7 also requires physical binding of the LPS core domain by its receptor tail prior to infection.

The two strains used in this study, *E. coli* K-12 substrains MG1655 and DFB1655 L9, are known to have equivalent growth rates and outer membrane compositions (6). MG1655 is a well characterized strain that also has a sequenced genome (8). DFB1655 is an isogenic derivative where DFB1655 L9 has a rescued *wbbL* gene which is required for the expression of O antigen on its surface (2). Therefore, any differences seen between these two strains is likely due to the expression of O16 antigen in DFB1655 L9.

Our results show that there is some mechanism of resistance to both T4 and T7 conferred by O16 antigen, as indicated by the lower susceptibility of the DFB1655 L9 strain to both T4 and T7 virus. This was displayed in both the growth curve and double agar overlay plaque assays. MG1655 was found to be susceptible to both T4 and T7 as indicated by phage mediated cell lysis, while DFB1655 L9 was shown to be resistant. However, the mechanism of resistance may not be due to our hypothesized model, in which O16 provides a physical barrier against bacteriophage from adsorbing to the core polysaccharides in LPS. Contrary to our expectations and the previous literature, our results from the adsorption assay detected less than 1 Cq difference between T4 and T7 concentration in the supernatant of MG1655 or DFB1655 L9 cells, compared to an average of 5 from Wachtel et. al., suggesting that there is no significant difference in T4 and T7 adsorption between substrains of MG1655 and DFB1655. While we used the same MOIs (with the exception of the lowest MOI which would not yield useful data) as conducted in the study by Wachtel *et al.,* and our experiment followed the same adsorption assay protocol that Wachtel *et al.* outlined in their experiment, but we were unable to reproduce the same results in our adsorption assay. This raises an interesting point of discussion where our study, and the work by Wachtel *et al.,* have shown contradicting results. It is possible that some undocumented differences in lab technique, or differences in the exact composition of reagents, would lead to differing results. We recognize that it is important for future experiments to repeat this adsorption assay (or perhaps investigate using

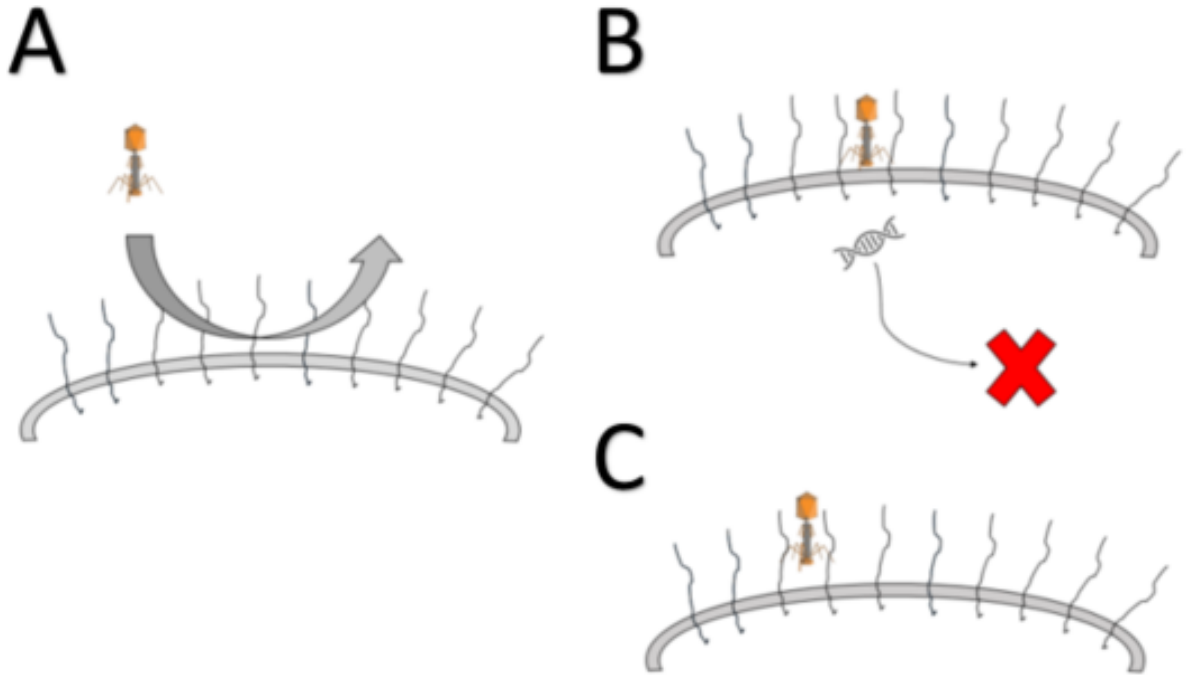

FIG. 6 Different possible mechanisms by which O16 antigen may confer resistance to *E. coli* DFB1655 L9 against T4/T7. Three possible models explaining how O16 antigen may confer resistance to T4 or T7 bacteriophage infection. **A)** Display of the steric hindrance model which we had first hypothesized as the most likely. O16 antigen is blocking phage from binding by steric hindrance, physically preventing it from binding to its receptor point, thereby inhibiting phage adsorption. **B)** Here, the O16 antigen somehow plays a role in blocking viral replication and subsequent infection after the viral DNA is ejected into the cell. However, this is an unlikely scenario as O16 antigen has not been shown to have any enzymatic activity and is located on the surface. **C)** Display of bacteriophage that has successfully adsorbed to the cell, but is unable to insert its DNA into the cell. It has therefore become 'trapped' on the surface of cell, preventing further infection.

a different method to measure adsorption) in order to obtain a significant result as to whether adsorption is blocked by O16 antigen. However, further discussion here will operate under the assumption that our results are not a product of laboratory failures and that O16 antigen does not block adsorption of T4 or T7 phage.

Our results indicate that O16 antigen confers resistance to T4/T7 mediated cell lysis, and so there are several different models which should be considered in understanding the mechanism behind this resistance. Chiu *et al.* incubated T4 in the supernatant of MG1655 and DFB1655 L9 overnight cultures to determine if there was a soluble component released from the cell into the supernatant which is responsible for neutralizing T4 (5). They found that there was no difference in infectivity between these bacteriophage compared to pure samples, indicating that a soluble forms of LPS inactivating T4 is an unlikely explanation for increased resistance in DFB1655 L9 (5).The cycle of infection for a bacteriophage starts with binding of the phage to the bacterial cell wall, followed by ejection of the phage genome into the cell, and finally replication/viral assembly/cell lysis once the viral DNA enters the cell (11). Given the know steps of viral infection, there are 3 general sites at which resistance against infection can be conferred. First, O16 antigen could prevent bacteriophage from binding to the cell, which is the steric hindrance model which Wachtel *et al.* proposed and is displayed in Figure 6A. Second, O16 antigen could result in bacteriophage binding to the surface of the cell such that the phage is unable to eject the viral genome into the cell, as displayed in Figure 6C. Third, any stage of viral replication once the viral genome successfully enters the cell could be blocked, as displayed in Figure 6B.

Because our data does not report any difference in T4 or T7 adsorption, our findings do not support the steric hindrance model proposed by Wachtel *et al.* If O16 was sterically hindering bacteriophage and preventing it from binding to the surface of the cell, then we would expect to have detected a higher concentration of phage in the supernatant of DFB1655 L9 cells compared to MG1655 in our adsorption assay. However, we did not see any difference between the quantities of phage left in the supernatant, suggesting that there was the same amount of T4 and T7 adsorption occurring in both MG1655 and DFB1655 L9. Furthermore, O antigen is located on the surface of the cell and has not been previously implicated to display any enzymatic capabilities, therefore it is unlikely that O16 antigen would be interfering with viral replication once the viral genome enters the cell. Given that these two mechanisms of resistance seem unlikely, the model of resistance that we propose to be most likely is where bacteriophage gets 'trapped' on the surface of the cell but is unable to eject its viral genome into the cell. Here, we would not see any differences in adsorption between MG1655 and DFB1655 L9 because T4 and T7 is still able to bind to the surface. However, upon binding, T4 and T7 would become inactivated such that it can longer continue its cycle of infection by ejecting its viral genome into the cell. This is consistent with our findings that there was no difference in adsorption between MG1655 and DFB1655 L9, yet DFB1655 L9 displayed resistance against both T4 and T7 whereas MG1655 was susceptible to infection. Because protein–saccharide interactions commonly involve stacking interactions between sugar residues and aromatic amino acid side chains, some O antigens have been previously implicated as binding receptors of bacteriophages (9). In T4 phage, host recognition occurs through a reversible interaction of the tip of the long tail fibers with LPS and OmpC. Upon receptor binding, another set of short-tail fibers extend and irreversibly bind to the outer core region of LPS (9). The latter interaction is necessary for ejection of viral DNA into the bacterium (9). If O16 contains a binding site for T4 phage positioned in such a way that a bound phage could not extend its short tail fibres to the LPS core region, either due to distance or the conformation of the O antigen physically blocking the phage receptor binding regions from reaching the receptor, then the cell would be effectively "trapped". T7 phage, while only possessing one set of tail fibers, also requires irreversible binding to the outer core region of LPS for ejection of viral DNA (10,12). The above mechanism would explain the identical results in T4 as well as T7.

In conclusion, our results support our hypothesis that O16 antigen is sufficient to confer resistance to T4 and T7 bacteriophage. Adsorption of the virus was tested via assay where qPCR quantifies the unabsorbed bacteriophage in the supernatant of the supernatant. Contrary to our expectations, there was no difference in T4 or T7 adsorption. Given this, our data does not support our hypothesis that O16 antigen blocks adsorption in T4 and T7 phage. This is

inconsistent with the previous findings by Wachtel *et al.* that observed high numbers of T4 bacteriophage in the supernatant of *E. coli* strain DFB compared to MG. Further experiments are needed to confidently determine whether O16 antigen prevents adsorption of bacteriophage T4 and T7.

Future Directions As previously mentioned, our results contradict the findings by Wachtel *et al.* in that we do not see reduced adsorption of T4 or T7. However, both studies performed the adsorption assay once. Therefore, one direction for future research is to perform several replicates of this adsorption assay in order to come to a meaningful conclusion as to whether or not O16 antigen reduces phage adsorption. Additionally, it would be interesting to pursue other methods of assessing adsorption to see whether this would produce similar results. Furthermore, it would be worth exploring other methods to assess other mechanisms conferring resistance between the two strains. For example, as presented in the discussion and displayed in Figure 6C, one possibility other than preventing adsorption is that O16 antigen leads to bacteriophage getting 'trapped' on the surface of the cell such that it is unable to eject its viral genome into the cell. An experiment that could be done to test this theory would involve inoculating MG1655 and DFB1655 L9 with T4 or T7 phage briefly, to allow adsorption to occur but not cell lysis. Next, both T4/T7 phage as well as the O16 antigen could be fluorescently stained and viewed with electron microscopy to observe the location of T4/T7 on the surface of the cell relative to O16 antigen. This model would predict that T4/T7 would be found bound to O16 antigen on the surface in DFB1655 L9 while it would be found right on the cell surface in MG1655.

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CONTRIBUTIONS

This project was a product of team effort between authors. Each author was involved in every step along the way, including writing the team proposal, brainstorming ideas, planning experiments, conducting experiments in the laboratory, writing and presenting the data, and writing the final paper. Major contributions **SL**: organizing, planning, troubleshooting, and executing experiments in the lab. **BB:** laying the foundation for the project through researching and compiling background information as well as main role in writing manuscript. **JN:** writing the team proposal, team presentation, and main role in writing of this manuscript. **JL:** managing timelines, maintaining and encouraging strong group communication, and assisting in laboratory experiments.

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