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Restoration of O-antigen production in *E. coli* K-12 DFB1655 confers resistance to T2 bacteriophage-mediated lysis

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SUMMARY Bacteriophage infection of *Escherichia coli* has long been studied to understand possible applications to bacterial disease, due to the specificity and efficiency of lysis. Commonly used strains such as *E. coli* K-12 lack expression of the outer membrane structure O-antigen, which is found in many wild-type strains. O-antigen expression impacts replication of bacteriophage reliant on interaction with host lipopolysaccharide. Previous studies using lipopolysaccharide-interacting T4 and T7 bacteriophage found that restored O-antigen expression in *E. coli* K-12 strain MG1655 did confer resistance to bacteriophage-induced lysis. However, it is unclear if this resistance is specific to T4 and T7 bacteriophage, or if resistance to other similar lipopolysaccharide-interacting bacteriophages is conferred. T2 bacteriophage has been demonstrated to take advantage of the lipopolysaccharide layer for adsorption in *E. coli* K-12 strains and exploit it for host cell entry through interaction with OmpF and FadL. We demonstrated that *E. coli* K-12 with restored O-antigen did not lyse when incubated with T2 bacteriophage through spectrophotometric bacterial growth curve assays and stab assays. Our findings provide preliminary results to suggest that O-antigen expression in *E. coli* provides resistance against lysis induced by lipopolysaccharide-interacting bacteriophage. Further understanding of these interactions may elucidate bacterial adaptations to bacteriophages and provide more context for industrial or therapeutic bacteriophage applications.

INTRODUCTION

Bacteriophage (phage) mediated viral infection of bacteria requires a high degree of specificity to the host species or bacterial strain. Phage specificity to bacteria is partially governed by the surface markers on bacteria that are used for viral binding and entry. These structures and their role in viral infection as well as viral infection resistance have not been well-characterized (1).

The O-antigen, or O-specific polysaccharides (OPS) is a polysaccharide molecule that can be present as part of the lipopolysaccharide (LPS) envelope that makes up the surface of Gram-negative bacteria (2). OPS plays a role in many bacterial functions, such as increasing colonization ability, bypassing host immunity, and providing protection against antibodies and antiviral compounds (3). In *Escherichia coli* K-12, OPS is synthesized by the *rfb* gene cluster, and consists of the *wbbI*, *wbbJ*, *wbbK*, and *wbbL* genes (4).

In the *E. coli* K-12 strain MG1655 (referred to as "MG1655") commonly used in lab environments, the ability to produce O-antigen has been lost (5). This is due to a loss of function mutation caused by a transposon insertion, named *rfb-50k* at the downstream end of the O-antigen *rfb* gene cluster at *wbbL* (5). The *wbbL* gene is used for rhamnosyl transferase activity in the growing O antigen polysaccharide chain (6). Browning *et al.* was able to use a suicide vector to repair functionality of the *wbbL* gene to generate a new strain: *E. coli* DFB1655 L9 (referred to as "DFB1655 L9") (4). O-antigen production in this *E. coli* K-12 strain is characterized by repeating D-N-acetylglucosamine, L-rhamnose, and D-galactofuranose subunits, with an O-acetyl side chain added on L-rhamnose. This particular configuration is denoted as O16 (6, 7). O16 has been shown to inhibit phage replication in types that target host LPS. Browning *et al.* had discovered that *E. coli* DFB1655 L9 had conferred resistance against phage P1 (4). In addition, other groups validated that DFB1655 L9 was able to also confer resistance against Enterobacteria phage T4 (T4 phage) infection (8, 9). A likely mechanism of this is through the prevention of phage adsorption into the host

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(7). Further investigations by other research groups have found similar resistance in strain DFB1655 L9 with T4 and Enterobacteria phage T7 (T7 phage) (8).

Other phages such as Enterobacteria phage T2 (T2 phage) are also known to infect *E. coli* and cause lysis first through interaction and adsorption via LPS, followed by infection via receptor proteins (10). While similar in structure to previously tested T4 phages, T2 phages differ in receptor protein function and target due to changes in amino acid sequences of the last 10% of the phage tail protein, which have been shown to radically alter receptor requirements (11). Past biochemical experiments and recent sequencing studies have shown T2 phage to preferably interact with surface proteins OmpF and FadL, differing from OmpC and OmpA/R for T4 and T7 phage respectively (10–13).

In this study, we had explored T2 phage resistance to determine whether O-antigen restoration in *E. coli* DFB1655 L9 confers resistance to phages other than T4 and T7 that target LPS. Consistent with previous literature studies conducted on T4, T7 and P1 phage which exploit LPS for entry into bacterial cells, we hypothesized that O-antigen production would provide resistance to T2 infection in a similar fashion due to the overall similarities of infection mechanism.

Screening of T2 phage for infectivity may provide insight into resistance mechanisms utilized by *E. coli* against phage, and the ability to generalize these observations to other phages that utilize LPS for adsorption. Furthermore, investigation into the role of O-antigen mediated phage resistance may be used to synthetically alter the microbial dynamics and community structure of industrial scale productions, as well as provide insight towards possible ramifications in phage therapy.

METHODS AND MATERIALS

Bacterial and phage strains. *E. coli* K-12 strains MG1655, DFB1655 L9 and BL21 laboratory stocks were retrieved from the Department of Microbiology and Immunology, University of British Columbia.

T4 phage stock was provided by the Department of Microbiology and Immunology at the University of British Columbia, originally obtained from the Carolina Biological Supply (cat no. 12-4330), and continuously propagated through previous experiments. T2 phage stock was obtained from the American Type Culture Collection (ATCC 11303-B2).

Isolation of strains and growing conditions for *E. coli* strains. *E. coli* strains MG1655 and BL21 were isolated by streaking of a single selected colony on 1.5% Luria broth (LB) agar plates, while DFB1655 L9 *E. coli* were grown on 1.5% LB agar plates supplemented with 50 μg/mL kanamycin. Cells were incubated at 37°C for growth. Single colonies were selected from plates to produce overnight cultures, which served as the working *E. coli* stocks for subsequent experiments.

PCR verification of *E. coli* strains. *E. coli* strains MG1655 and DFB1655 L9 were allowed to grow overnight at 37°C in LB broth and LB supplemented with 50 mg/ml kanamycin respectively. Genomic DNA (gDNA) was extracted from 5 mL of each overnight culture using the PureLink Genomic DNA kit (ThermoFisher) according to manufacturer's instructions. The concentration and quality of extracted gDNA was confirmed by the NanoDrop 2000 spectrophotometer.

Extracted gDNA served as a template. Primers were designed based on the wbbL primers used for cloning by Browning *et al.* (4) . The overhangs were removed for our PCR validation. To confirm the identity of *E. coli* K12 strains MG1655 and DFB1655 L9, the restored *wbbL* gene in DFB1655 L9 and the *wbbL* containing the insert sequence in MG1655 were amplified (Table 1). Primers were used at a final concentration of 0.2 μM, and 250 ng of each gDNA sample was added to the PCR reaction with Taq DNA Polymerase (ThermoFisher) to a total final volume of 50 μL per reaction. A reaction using 2 μL of sterile water instead of template DNA was used as a negative control.

The Bio-Rad T100™ Thermal Cycler was programmed as follows: Initial denaturation for 5 minutes at 95 C, then 30 cycles of: denaturation for 30 seconds at 95 C, annealing for 45 seconds at 55 C, elongation for 2 minutes at 72 C. After 30 cycles, a final elongation at 72 C for 5 minutes is performed followed by storage at 4 C indefinitely. PCR products were

TABLE 1 *E. coli* K-12 strain *wbbL* primers and T4, T2 phage *gp23* primers for validation. F and R represent forward and reverse primers respectively.

Target	Sequence (5' - 3')	Expected Size (bp)
MG1655 *wbbL*	F: ATGGTATATATAATAATCGTTTCCC R: TTACGGGTGAAAAACTGATG	1994
DFB1655 L9 *wbbL*	F: ATGGTATATATAATAATCGTTTCCC R: TTACGGGTGAAAAACTGATG	799
T4 *gp23*	F: GCCATTACTGGAAGGTGAAGG R: TTGGGTGGAATGCTTCTTTAG	398
T2 *gp23*	F: CTCAGGCATTCGGTTCTTTC R: TGTAAATAGTTCCGACTTCAAGAG	369

visualized on 1% agarose gel with 1x TAE at 100V for 50 minutes via the addition of 1X working RedSafe Nucleic Acid Staining Solution (FroggaBio). Products were compared against a 1 Kb Plus DNA ladder (Invitrogen). Gels were imaged using a Chemidoc Imaging System (Bio-Rad).

Propagation and isolation of T4 and T2 phage. To propagate T4 phage, overnight cultures of MG1655 were diluted in a 1:5 ratio in 10 mL of LB broth supplemented with 1 mM $CaCl_2$. After dilution, 20 µL of T4 phage stock was added and allowed to replicate within the culture overnight or until cleared on a shaking platform at 37°C. Remaining cells in cultures were lysed by adding 200 µL of chloroform and shaken for 5 minutes at room temperature. Subsequently, cell debris was spun down at 4000 g for 10 minutes at room temperature. The supernatant was then filtered through a 0.45 µM syringe filter to ensure sterility, and stored at 4 C. To propagate T2 phage, the same procedure was completed with *E. coli* strain BL21 instead of MG1655.

PCR identity confirmation of T4 and T2 phage. T4 phage isolate and T2 isolate were directly used as a template for amplification of the *gp23* gene present in both T4 and T2 phage. T4 phage primers from Chiu *et al.* (14). were used to confirm the identity of T4 phage via amplification of a 398 bp region, while T2 phage primers were designed to amplify a 369 bp region. Primers were used at a final concentration of 0.2 µM, and 1 µL of phage lysate was added to the reaction to provide template DNA. A negative control was run using 2 µL of sterile water instead of template DNA.

The Bio-Rad T100™ Thermal Cycler was programmed as follows: Initial denaturation for 5 minutes at 95 C, then 30 cycles of: denaturation for 30 seconds at 95 C, annealing for 45 seconds at 62 C, elongation for 1 minute at 72 C. After 30 cycles, a final elongation at 72 C for 5 minutes is performed followed by storage at 4 C indefinitely. PCR products were visualized on 1% agarose gel with 1x TAE at 100V for 50 minutes via the addition of 1X working RedSafe Nucleic Acid Staining Solution (FroggaBio). Products were compared against a 100bp Plus DNA ladder (Invitrogen). Gels were imaged using a Chemidoc Imaging System (Bio-Rad).

Stab assay to assess differential susceptibility of MG1655 and DFB1655. To perform the stab assay, 100 µL of overnight culture of MG1655 or BL21 was spread over a LB agar plate supplemented with 1 mM CaCl2 using a sterile glass spreader until dry. Similarly, 100 µL of DFB1655 L9 was spread over a LB agar plate with the addition of 50 µg/mL kanamycin and 1 mM CaCl2. For each plate, the surface area was split in half. To serve as a positive control, 5 µL of T2 phage stock (ATCC 11303-B2) was dropped at four individual locations on the left half. On the right half, 5 µL of T2 phage isolate was dropped four times to assess susceptibility of MG1655 versus DFB1655. BL21 plates served as controls, as susceptibility to lysis by T2 phage in this strain was established during propagation.

T4 and T2 phage OD assays to assess differential susceptibility of MG1655 and DFB1655 L9 to infection. A master mix of LB and 1 M $CaCl_2$ stock was made at a ratio of 1000:1.

Next, 150 µL of media was pipetted into each well with 50 µL of overnight culture of the desired *E. coli* strain. For each specified phage stock, 2 µL was added to the wells. Experiments were run in triplicates with each *E. coli* strain (MG1655, DFB1655 L9, and BL21) inoculated with either no phage, T2 phage or T4 phage. Wells containing only 200 µL of LB media were used as blanks. Plates were incubated at 37°C, and continuously shaken at 200 rpm to ensure aeration throughout the measurement process. At intervals of 10 minutes, OD_{600} readings were taken in each well over a 12 hour time period using the Epoch 2 Microplate Spectrophotometer (BioTek), to a total of 48 data points per well. Results were visualized using Graphpad Prism 9.0.0.

Plaque assay methods. The following methods are adapted from Wachtel *et al.*, and Bremner *et al.* (8, 15). For enumeration of T4 and T2 phage within the generated lysate, *E. coli* strain BL21 was used.

For each phage stock, serial dilutions of 10^{-1} to 10^{-11} were performed by adding 100 µL of phage stock to 900 µL of LB consecutively. To plate each dilution, 100 µL of the diluted stock and 100 µL of overnight *E. coli* BL21 culture was added to 3 mL of warmed, liquid 0.7% LB agar supplemented with 1 mM CaCl2. The 0.7% agar was then poured over standard LB agar plates with added 1mM CaCl2. Plates were set for 15 minutes at room temperature, then incubated at 37°C for 4-6 hours. Each dilution was plated in duplicate. Each observed plaque was presumed to be an individual plaque forming unit (PFU).

RESULTS

PCR verification of *E.coli* K12 strains confirmed a disrupted *wbbL* gene in MG1655 and a rescued *wbbL* in DFB1655 L9. MG1655 and FB1655 L9 strain identities were validated by confirming the status of *wbbL* via PCR. Genomic DNA was isolated from each strain and *wbbL* was amplified. MG1655 contains an IS5 transposon insertion in *wbbL* and therefore the gene has an expected size around ~2kb (4). Previous work cloned *wbbL* from *E. coli* K12 WG1 into suicide vector pJp5603 . This was then integrated into the chromosomal *wbbL* locus of MG1655 to create DFB1655 L9, which resulted in loss of the integrated transposon insertion and restored O-antigen production. The expected band size for DFB1655 L9 *wbbL* was 799 bp.

PCR amplification of MG1655 and DFB1655 L9 *wbbL* yielded a 2000 bp and 800 bp product respectively as expected (FIG. 1A). DFB1655 L9 also showed two faint bands around ~2000 bp and ~1500 bp. No bands were observed in the no-template control condition.

T2 and T4 bacteriophage identity confirmation through PCR amplification of *gp23* with two sets of primers. To validate our bacteriophage strains, PCR amplification of the *gp23* capsid protein gene was executed. The first primer set (T4 primers) was obtained from a previous study (8) and found to amplify both T4 and T2 *gp23* at a product size of 398 bp (Table 1). To differentiate between the two phages, we designed a second set of primers specific to T2 *gp23* with a product size of 369 bp (Table 1). T4 and T2 lysate were amplified with either T4 primers or T2 primers in duplicates. Amplification of T4 and T2 *gp23* with T4 primers each yielded a 400 bp product as expected (FIG. 1B). Additionally, amplification of T4 and T2 *gp23* with T2 primers yielded no product and a smaller < 400 bp product respectively. Negative control lanes did not contain any bands (FIG. 1B). From these results, we successfully confirmed the identity of the two phages and that no contamination occurred between the two lysates.

***E. coli* DFB1655 L9 exhibited resistance to lysis by T2 bacteriophage.** To verify DFB1655 L9 resistance and MG1655 susceptibility to T4 and T2 phage, an 8 hour OD_{600} time series was conducted and measurements were taken at 10 minute intervals. The MG1655, DFB1655 L9 and BL21 no phage controls all show the same pattern of growth exhibiting significant increases in absorbance before leveling off after 8 hours (FIG. 2A). MG1655 inoculated with T4 phage showed an initial increase in OD_{600} until 50 minutes before rapidly falling and remaining static at an OD_{600} of 0.05. Similarly, BL21 inoculated with T4 phage showed an increase and decrease in OD_{600} over the time series. DFB1655 L9 inoculated with T4 phage, as expected, follows the same pattern as the control group. MG1655 and BL21 inoculated

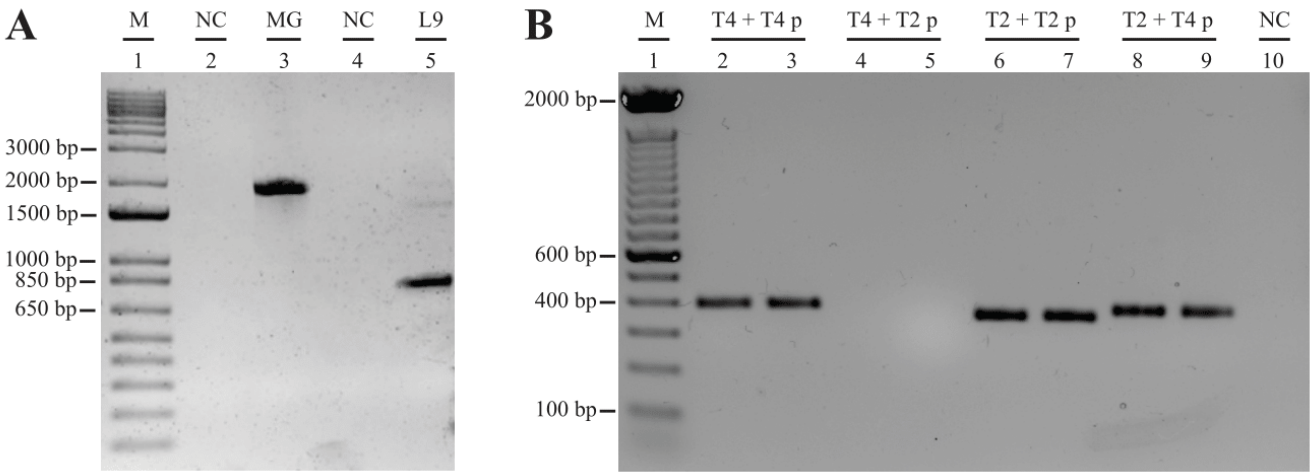

FIG. 1 PCR amplification of *E. coli* and bacteriophage strains shows the expected bands for each condition. (A) PCR was performed on MG1655 (Lane 3) and DFB1655 L9 (Lane 5) isolated genomic DNA with *wbbL* specific primers. Negative controls were run with no template (Lanes 2 and 4). **(B)** PCR was performed on T4 phage lysate with T4 primers (Lanes 2 & 3) or T2 primers (Lanes 4 & 5) and T2 phage lysate with T2 primers (Lanes 6 & 7) or T4 primers (Lane 8 & 9). Negative control was run with no template (Lane 10). PCR products were run on a 1% agarose gel. M = Marker; NC = Negative Control; MG = MG1655; L9 = DFB1655 L9; T4 p = T4 primer; T2 p = T2 primer.

with T2 phage showed unexpected results; there was an initial increase in OD_{600} over a longer period, followed by a small drop in OD_{600} where it remained static compared to T4 phage inoculated MG1655 which resulted in a drastic decrease in OD_{600}. DFB1655 L9 inoculated with T2 phage resulted in a similar pattern of uninhibited growth as the no phage controls.

Similar results were observed for the stab assay conducted. Both T2 phage stock and T2 phage isolate were able to lyse bacterial lawns of BL21 and MG1655, exhibiting visual zones of clearance, while no plaques were observed on the DFB1655 L9 lawn (FIG. 2B-D). Taken in conjunction with the results of the OD_{600} assay, this confirms that DFB1655 L9 exhibits resistance to T2 phage.

Plaque assays successfully quantified T4 and T2 phage isolate concentration. Phage concentrations in PFU/mL were enumerated using serially diluted plaque assays. Countable plaques were visible on the 10^{-8} dilution of T2 phage isolate, and duplicates were used to retrieve a concentration of 5.5×10^{10} PFU/mL (FIG. 3A, B). For the T4 phage isolate, countable plaques were observed at dilution of 10^{-11} and duplicate plates were used to calculate a concentration of 9.9×10^{13} PFU/mL (FIG. 3C, D). DFB1655 L9 plates incubated with undiluted T4 and T2 phage yielded no plaques (FIG. 3E, F).

DISCUSSION

The purpose of this study was to elucidate whether restoration of O16 antigen in DFB1655 L9 provided resistance to T2 bacteriophage in a similar manner previously shown in T4 bacteriophage. To achieve this, we first validated the identity of our *E.coli* K-12 and bacteriophage strains. PCR amplification of the *wbbL* gene in both K-12 strains showed a 2 kbp product in MG1655, indicative of the IS5 transposon insertion. In contrast, the product in DFB1655 L9 lacked this IS5 transposon insertion, and we visualized an 800 bp product as a result. We also observed two faint bands in the rightmost lane corresponding to around 1.5 kbp and 2 kbp. These bands have been observed in previous studies and are likely due to non-specific binding to residual *wbbL* containing IS5 in the genome of DFB1655 L9 after vector integration respectively (4, 8, 14).

By conducting an OD_{600} time series experiment, we aimed to investigate the infection kinetics of T2 lytic infection. The results corroborated the findings of the plaque assays with no observable difference in OD_{600} measurements in DFB1655 L9 strains inoculated with T2, T4 phage and no phage. Comparatively, there was significant lytic activity observed in the wells containing MG1655 *E. coli* inoculated with T4 and T2 phage. However, T2 phage

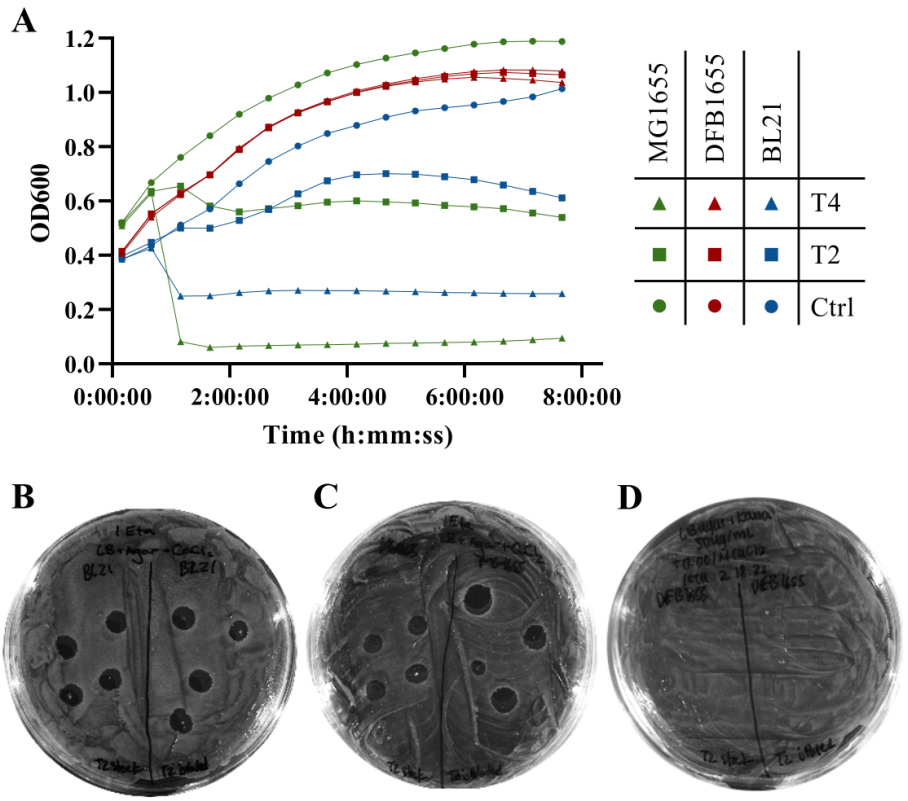

FIG. 2 T2 phage inhibits MG1655 growth but not DFB1655 L9. (A) Overnight cultures of MG1655 (green), DFB1655 L9 (red), and BL21 (blue) were diluted 1:3 and incubated at 37°C with T4 (triangle), T2 (square), or no phage (circle) in a 96-well plate. OD_{600} was measured every 10 minutes over the course of 8 hours. Each condition was performed in triplicate. Lawns of BL21 **(B)**, MG1655 **(C)** grown on LB agar plates supplemented with 1 mM $CaCl_2$, and DFB1655 L9 **(D)** grown on LB agar plates with 50 µg/mL kanamycin and 1mM CaCl2 were incubated overnight at 37°C with 2 µL of T2 phage stock (left half of each plate) or T2 phage isolate (right half of each plate) at four locations.

appeared to only inhibit growth initially and required much longer to lyse the cells compared to T4. Interestingly, there was a slight increase in OD_{600} measurements past 600 minutes for MG1655 and BL21 inoculated with T4 phage. The same pattern was not observed in MG1655 and BL21 inoculated with T2 phage. Chiu *et al.* suggests that this increase is due to the natural development of phage resistance via a separate unknown mechanism (14). It is possible that T2 phage is unable to conduct complete bacterial lysis compared to T4 phage due to different invasion mechanisms or virulence factors that could be explored further.

While we aimed to investigate the differential susceptibility to T2 phage infection in DFB1655 L9 and MG1655, we also observed partial resistance to T2 phage infection in the *E. coli* BL21 strain. T2 inoculated BL21 resulted in an increasing OD_{600} measurement over the 8h incubation. Strikingly, BL21 inoculated with T2 showed higher OD_{600} measurements over the 8h time series compared to BL21 inoculated with T4 phage. BL21 does not contain Ion protease as well as the outer membrane protease OmpT (16). These proteases are involved in the degradation of heterologous protein and may play a role in the resistance observed from the OD_{600} time series.

Phage isolate concentrations for both T4 and T2 phage were quantified using plaque assays, which are considered the “gold standard” for phage enumeration (17). We found that T2 phage plaques were uncountable on lawns of *E. coli* MG1655 due to extremely small plaque sizes. To account for possible differences in adsorption or other differences between MG1655 and BL21 strains that may impact enumeration, both T4 and T2 bacteriophage were quantified by co-inoculating with *E. coli* strain BL21.

Interestingly, we observed differential results from the growth curves compared to the plaque assays. Plating of T2 phage isolate on lawns of MG1655 produced small plaques that were uncountable as a result of their size (not shown). This is in contrast to our OD_{600} assay results, as we observed similar growth and lysis dynamics between MG1655 and BL21 when incubated with T2 phage isolate. We hypothesize that differences observed between lysis dynamics in MG1655 and BL21 in the OD_{600} assay and plaque assays may be due to aeration, growth on plates versus in wells, or other inconsistencies in growth conditions between the two experiments and could be investigated in future experiments to understand possibly confounding variables.

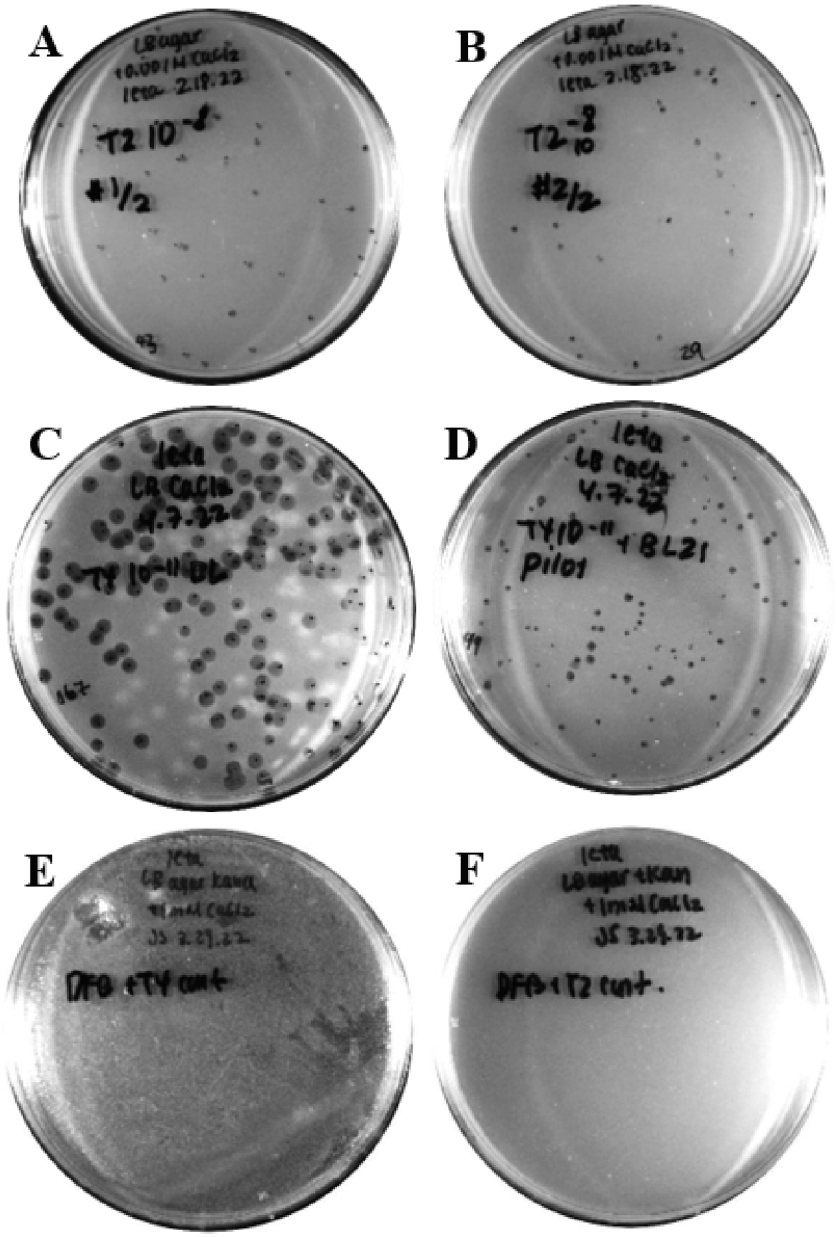

FIG. 3 Plaque assays with *E. coli* BL21 quantified phage concentration in T2 and T4 phage isolate. Lawns of BL21 were grown on LB agar plates supplemented with 1 mM CaCl2, and incubated for 4-6 hours at 37°C. Countable plaques were observed at dilutions of 10^{-8} for T2 phage isolate **(A, B)**. At dilutions of 10^{-11}, countable plaques were visible for T4 phage isolate **(C, D)**. DFB1655 L9 with undiluted T4 and T2 served as controls **(E, F)**. Each dilution was plated in duplicate.

T4 phage has been well-described in recent years and is understood to have a double-stranded, linear DNA genome. T4 phage has endonucleases that play a role in breakdown of host DNA to usable nucleotides for phage replication. T4 phage has also been characterized to possess the ability to modify their own DNA as a method to protect phage DNA from degradation via self-endonucleases (18). Other phages such as T2 are known to infect *E.coli* and have been characterized to invade via interaction with the LPS layer of *E.coli*. T2 phage differs from T4 phage in its target protein receptors due to changes in the amino acid sequences of the phage tail proteins. Changes to the phage tail proteins have been demonstrated to largely impact the receptor requirements of the phage (11). Literature suggests T2 phage shows preferential interaction with OmpF and FadL compared to OmpC and OmpA/R for T4 phage. Thus, it is possible that the presence of these receptors may play a role in the infection kinetics of both T4 and T2 phage.

This study describes the differential susceptibility of MG1655 and DFB1655 L9 to T2 phage infection. Previously reported to be resistant to T4 and T7 phage as a result of O-antigen production in DFB1655 L9 (19), we provide the first evidence that this strain is also resistant to T2 phage. Although our results showed that MG1655 is susceptible to T2 phage,

growth curve analysis revealed a large distinction between MG1655 incubated with T4 versus T2 phage, with the latter requiring longer incubation times for only incomplete lysis. While we were able to characterize the differential susceptibility of MG1655 and DFB1655 L9, this provides a basis of knowledge for similar O-antigen serotypes to be studied.

Limitations Due to small plaque sizes after incubation, we were unable to enumerate T2 phage isolate with MG1655. It may be possible that BL21 and MG1655 differ in susceptibility to T2 phage, making the enumeration performed in this study only applicable to *E. coli* strain BL21. When conducting the OD_{600} time series, further investigation may benefit from standardizing the concentrations of phage used. Smaller or larger concentrations of phage inoculated into each *E. coli* strain may affect infection kinetics and standardizing these concentrations may reduce the chance of introducing potential confounding variables. Furthermore, due to technical and time constraints, T2 and T4 phage enumeration plaque assays were only plated in duplicate. As a result of the small sample size, we lacked the power to make any statistical comparisons.

Conclusions In conclusion, we employed OD_{600} assays and stab assays to confirm differential susceptibility of MG1655 and DFB1655 L9 to T2 phage infection and subsequent lysis. Our hypothesis of O-antigen expression preventing infection of T2 phage was supported, finding that incubation of MG1655 with T2 resulted in decreased OD_{600} and visible plaques, while DFB1655 L9 incubated with T2 under identical conditions did not show the same susceptibility. These results suggest that O-antigen expression in DFB1655 L9 confers resistance to lysis by T2 phage.

Future Directions Throughout the course of this experiment, we investigated the differential susceptibility of MG1655 and DFB1655 to T2 phage. However, we also observed differential susceptibility of MG1655 and BL21 to infection and lysis by T2 phage during the propagation and OD_{600} assays involving T2 phage. Further studies could elucidate the difference between these *E. coli* strains and possible explanations for this observation, as BL21 is known to lack the OmpT surface protein found in K-12 strains, as well as other modifications to the genome (20).

Furthermore, while previous studies have hypothesized that the mechanism of resistance is a result of O-antigen preventing adsorption to the LPS, other models of resistance have also been proposed (7–9). Our experiments demonstrate resistance to T2 phage-induced lysis in DFB1655 L9, but do not differentiate between the possible mechanisms of resistance. Subsequent studies may wish to employ methods such as fluorescence microscopy, qPCR adsorption assays, or transmission electron microscopy to further understand the basis of resistance to T2 phage lysis in DFB1655 L9.

Finally, while this study provides a basis for how T2 phage interacts with *E. coli* strains MG1655, DFB1655 L9 and BL21, future investigations may evaluate whether resistance is linked to the O-antigen specific structure. Phages other than the previously investigated T4, T7, P1, and T2 that use LPS structures to invade host cells could be screened for infectivity. Subsequent studies may further elucidate the phage-host interactions by visualizing the interactions between T2 phage and the *E. coli* strains and may provide more context to the mechanism of resistance that was observed in this study. While we have demonstrated T2 phage resistance in DFB1655 L9 is likely due to O-antigen expression, it only provides a basis of knowledge for potential future practical applications.

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CONTRIBUTIONS

This project was completed by IA, AC, JS and KX. Authors collaborated for each aspect of the project: drafting proposals and summaries, doing background research, preparing and ordering laboratory reagents, conducting experiments, collecting and visualizing data, and drafting the manuscript. IA contributed to background research, assisting laboratory experiments, drafting and revising figures, methodology and results sections. AC contributed to background research, assisting in laboratory experiments, and drafting abstract, future directions, results and discussion sections. JS contributed to general laboratory and experimental work, outlining and drafting abstract, introduction, limitations, conclusions, methodology and results section. KX contributed to brainstorming, troubleshooting and conducting laboratory experiments, generating manuscript figures and captions, drafting and revising the methodology, results and discussion sections of the manuscript. Authors contributed equally to formatting, retrieving citations, document flow and general proofreading.

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