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Testing the effect of a *wbbI* knockout on T4 bacteriophage resistance in *Escherichia coli* K-12

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SUMMARY Since its isolation, *Escherichia coli* K-12 has been used as a model organism and has become a common laboratory strain. In the outer membrane of *E. coli*, the O16 antigen is attached to the core sugars in lipopolysaccharide. The O16 antigen is synthesized by the WbbL, WbbK, WbbJ, and WbbI proteins encoded by the *rfb* cluster. Previous studies have shown that strains that possess a functional *wbbL* and express the O16 antigen demonstrate resistance to T4 bacteriophage, while strains that do not express the O16 antigen due to a disrupted *wbbL* gene are susceptible. However, the mechanism of this resistance remains unknown. Knowing that WbbI catalyzes the linkage of the distal sugar, D-galactofuranose, to the O16 antigen, the goal of this study was to determine the effect of a *wbbI* knockout on T4 bacteriophage resistance. Previous mechanisms have proposed that T4 tails attach to D-galactofuranose of the O16 antigen, which hinders the attachment of the T4 tails to the OmpC receptor. Thus, we hypothesized that the absence of D-galactofuranose on the O16 antigen as a result of a *wbbI* knockout would decrease resistance to T4, since attachment is no longer hindered by the O16 antigen. To investigate our hypothesis, we cloned *wbbL* back into JW2019-1, which contains a *wbbI* knockout as well as a disrupted *wbbL* gene, such that it expressed the truncated O16 antigen. We tested the *wbbI* knockout strain for T4 bacteriophage susceptibility via a stab assay. However, because our controls did not demonstrate the expected resistance phenotype to T4 bacteriophage, we were unable to determine the effect of a *wbbI* knockout on T4 bacteriophage resistance.

INTRODUCTION

The Gram-negative *E. coli* K-12 strain is surrounded by an outer membrane composed of phospholipids in the inner leaflet and lipopolysaccharides (LPS) in the outer leaflet (1). LPS is a glycolipid that consists of lipid A linked to a core of sugar units (1). Attached to the core sugars is an oligosaccharide unit called the O antigen that has been classified into more than 180 variants (2). O16 is an O antigen variant that is synthesized by proteins encoded in the *rfb* cluster (1). One of the genes in the cluster, *wbbL*, encodes for a rhamnose transferase that catalyzes the covalent linkage between the first two sugars in the O antigen, D-N-acetylglucosamine and L-rhamnose (3). After WbbL adds L-rhamnose, the other proteins encoded on the *rfb* cluster add the remaining sugars onto the growing O antigen (3). WbbK adds D-glucose onto L-rhamnose, WbbJ adds O-acetyl to L-rhamnose, and WbbI adds D-galactofuranose to D-glucose (3).

Genetic lesions caused by repeated passaging and mutagen exposure have resulted in the loss of O16 antigen expression in *E. coli* strain MG1655 due to the disruption of the *wbbL* gene by an IS5 transposon insertion element (1, 3). The expression of the O16 antigen was restored in MG1655 by complementing *wbbL* on a plasmid, resulting in the DFB1655 L9 strain (1).

MG1655 exhibited susceptibility to T4 bacteriophage whereas DFB1655 L9 showed resistance to T4 bacteriophage owing to the restored O antigen as demonstrated by Dimou et al. in a phage adsorption assay (4). Morgan *et al*. utilized transmission electron microscopy to demonstrate that MG1655 had notably more T4 bacteriophage attached to its surface compared to DFB1655 L9 (5). Further, using a lytic assay to determine T4 bacteriophage susceptibility, Morgan *et al*. observed a decrease in the OD_{600} of MG1655 in the presence of T4 bacteriophage due to bacteriophage-induced lysis (5). However, the OD_{600} of DFB1655

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L9 in the presence of T4 bacteriophage remained constant, suggesting that bacteriophage-induced lysis was inhibited or hindered (5). While the mechanism by which the O16 antigen mediates resistance is unknown, one proposed mechanism suggests that the O antigen chain may sterically hinder the attachment of T4 bacteriophage to the *E. coli* osmoregulator OmpC (6, 7). This prevents viral infection of *E. coli* with T4 bacteriophage (6).

Since WbbI catalyzes the final step in O antigen synthesis, we aimed to determine the effect of a *wbbI* knockout and truncated O antigen on T4 resistance. We hypothesized that the absence of D-galactofuranose from the O16 antigen as a result of the *wbbI* knockout would result in decreased resistance to T4 bacteriophage, as the truncated O16 antigen will no longer hinder T4 tails from attaching to OmpC. To test our hypothesis, we utilized the *E. coli* strain JW2019-1 which contains a *wbbI* knockout as well as a disrupted *wbbL* gene. We cloned *wbbL* back into JW2019-1, such that we generated a bona fide *wbbI* knockout that expressed a truncated O16 antigen lacking the terminal sugar moiety, D-galactofuranose. We then performed stab assays to determine the susceptibility of *E. coli* K-12 to T4 bacteriophage as a result of the *wbbI* knockout. Zones of lysis were observed after overnight incubation (8). The stab assay results for JW2019-1 transformed with *wbbL* demonstrated susceptibility to T4 bacteriophage. To ensure that the observed phenotype in transformed JW2019-1 was due to the *wbbI* knockout, we attempted to restore O16 antigen expression in MG1655 by transforming *wbbL* into this strain. However, the stab assay results indicated that transformed MG1655 failed to demonstrate the expected resistance phenotype to T4 bacteriophage. Since the control failed and the stab assay yielded inconclusive results, we were unable to conclude that the susceptibility observed in transformed JW2019-1 was due to the *wbbI* knockout alone.

METHODS AND MATERIALS

Bacterial and bacteriophage strains. *E. coli* strains MG1655, DFB1655 L9, JW2019-1, and WG1, as well as T4 bacteriophage were obtained from the lab stocks in the Department of Microbiology and Immunology at the University of British Columbia. Bacterial strains were grown on Luria-Bertani (LB) agar plates with the appropriate antibiotics, incubated at 37ºC overnight, then stored at 4ºC.

T4 bacteriophage propagation. An overnight culture of MG1655 was subcultured by performing a 1:100 dilution in LB broth supplemented with 0.001M $CaCl_2$ and $MgCl_2$. The subculture was incubated for 1 hour at 37ºC with shaking at 200 RPM. Following the incubation period, 100µL of T4 bacteriophage stock was added to the culture and incubated overnight at 37ºC with shaking at 200 RPM. The following day, the culture was centrifuged at 4,000 x g for 20 minutes and the supernatant containing bacteriophage was collected. The supernatant was filter-sterilized through a 0.22µm pore and stored at 4ºC.

Genomic DNA Extraction, PCR amplification, and gel electrophoresis for confirming strains. Genomic and plasmid DNA were isolated from overnight cultures of MG1655 and DFB1655 L9 using the PureLink Genomic DNA kit (Thermo Fisher). Touchdown and gradient PCR was performed using Platinum SuperFi DNA Polymerase (Invitrogen) and primers designed by Browning *et al.* (Table 1) to amplify both the restoration of *wbbL* on a

TABLE. 1 Primers designed for amplifying *wbbL* and pUC19 in *E. coli* K-12 strains.

Gene	Sequence (5' – 3')	Tm (°C)
wbbL	F: CCCGAATTCATATGGTATATATAATAATCGTTTCCC	58
	R: CCCAAGCTTCTCGAGTTACGGGTGAAAAACTGATGAAATTC	65.4
pUC19	pUC19-193F: GTGAAATACCGCACAGATGC	54.3
	pUC19-355R: GGCGTTACCCAACTTAATCG	55.6

plasmid in DFB1655 L9 and *wbbL* containing the IS5 insertion in MG1655 and DFB1655 L9 (1). The PCR was performed according to the parameters outlined in Table 2. pUC19 plasmid DNA was used as a positive control and was amplified using primers shown in Table 1, while water was used as a no template control. Gel Loading Dye, Purple (6X) (New England BioLabs) was added to PCR products. Then, PCR products with Gel Loading Dye were run on a 1% agarose gel in 1X TAE with a 1 Kb Plus DNA ladder (Thermo Fisher Scientific) and visualized with RedSafe Nucleic Acid Staining Solution (iNtRON Biotechnology) using the Bio-Rad ChemiDoc.

TABLE. 2 PCR parameters to amplify the *wbbL* gene.

PCR step	Temperature	Time	Number of Cycles
Initial denaturation	95°C	4 min	1
Denaturation	95°C	30 sec	6
Annealing	61°C, -1°C per cycle to 53°C	45 sec	
Extension	75°C	2.5 min	
Denaturation	95°C	30 sec	28
Annealing	53°C	45 sec	
Extension	75°C	2.5 min	
Final extension	75°C	10 min	1

Cloning *wbbL* into the pCR™4-TOPO® vector, sequencing, and transforming TOP10 Chemically Competent cells. TOP10 Chemically Competent cells transformed with the plasmid containing *wbbL* that we used throughout this project were obtained from our colleagues in the MICB401 lab. Our colleagues performed the subsequent cloning methods to generate the plasmid containing *wbbL*.

Genomic DNA was isolated from an overnight culture of WG1 using the PureLink Genomic DNA kit (Thermo Fisher). PCR was performed using the Maxima Hot Start Taq DNA polymerase (Thermo Fisher) and primers designed by Browning *et al.* (1) to amplify *wbbL* in WG1 (Table 1). PCR was performed according to the parameters outlined in Table 2. pUC19 plasmid DNA was used as a positive control and was amplified using primers shown in Table 1, while water was used as a no template control. Gel Loading Dye, Purple (6X) (New England BioLabs) was added to PCR products (New England BioLabs). Then, PCR products with Gel Loading Dye were run on a 1% agarose gel in 1X TAE with a 1 Kb Plus DNA ladder (Thermo Fisher Scientific), and visualized with RedSafe Nucleic Acid Staining Solution (iNtRON Biotechnology) using the Bio-Rad ChemiDoc.

The cloning reaction was performed according to the methods outlined in the TOPO® TA Cloning® Kit for Sequencing (Invitrogen) using 4µL of *wbbL* PCR product to produce the pCR™4-TOPO®-*wbbL*+ plasmid construct (Fig. 1) (9). To confirm the insertion of *wbbL* into the vector, Sanger sequencing was performed on pCR™4-TOPO®-*wbbL*+ (Genewiz). The results were aligned to the reference genome using NCBI Blast.

pCR™4-TOPO®-*wbbL*+ was transformed into TOP10 Chemically Competent cells according to the methods outlined in the TOPO® TA Cloning® Kit for Sequencing (Invitrogen) (9). Blue-white colony screening on LB plates containing 50µg/mL ampicillin and 20µg/mL of X-gal was used to confirm that *wbbL* was inserted into the *LacZα-ccdB* gene of the pCR™4-TOPO® vector. White colonies denoted those with the *wbbL* insertion into the pCR™4-TOPO® vector.

Preparation of competent MG1655 and JW2019-1 cells. Preparation of competent cells was performed as described by Chang *et al*. (10). MG1655 and JW2019-1 overnight cultures were subcultured by performing a 1:100 dilution into LB broth. The subcultures were then incubated at 37ºC with shaking at 200 RPM until the OD_{600} reached 0.4. Cells were incubated on ice for 20 minutes, and centrifuged at 4ºC at 4000 RPM for 10 minutes. Cells were then resuspended in 20mL ice-cold 0.1M $CaCl_2$ and incubated on ice for 30 minutes. Next, cells were centrifuged at 4ºC at 4000 RPM for 10 minutes, resuspended in 20mL ice-cold 0.1M

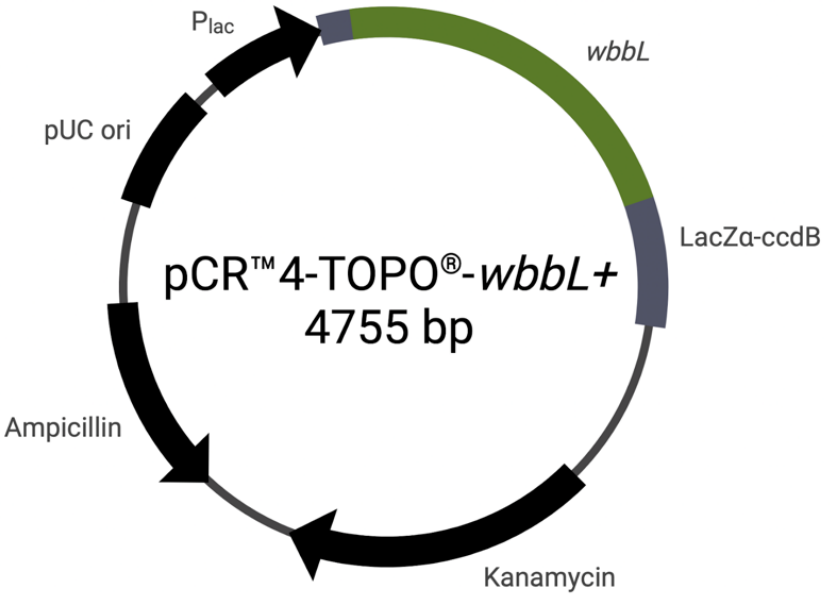

FIG. 1 pCR™4-TOPO®-*wbbL+* plasmid construct. The lac promoter, pUC origin of replication, ampicillin resistance gene, kanamycin resistance gene, and the *wbbL* gene inserted into the *LacZα-ccdB* sequence are shown.

$CaCl_2$, then incubated again on ice for 30 minutes. Cells were centrifuged at 4ºC at 4000 RPM for 10 minutes and resuspended in 2.5mL ice-cold 0.1M $CaCl_2$ supplemented with 15% glycerol. Finally, competent cells were stored at -70ºC.

Heat shock transformation and plating transformed cells. The BioBasic EZ-10 Spin Column Plasmid DNA Miniprep Kit was used to extract pCR™4-TOPO®-*wbbL+* from TOP10 Chemically Competent cells (11). Heat shock transformation was performed as described by Chang *et al*. (10). 6µl of 100pg/µl of pCR™4-TOPO®-*wbbL+* was added to 75µl of competent MG1655 or JW2019-1 cells. Cells were then incubated on ice for 30 minutes, followed by a 30 second heat shock treatment at 42ºC in a water bath, and a 2-minute incubation on ice. 1mL of pre-warmed LB broth was added to each tube of transformed cells, followed by a 1 hour outgrowth step at 37ºC and 200 RPM. The pCR™4-TOPO®-*wbbL+* was used in two separate transformation reactions with MG1655 or JW2019-1 competent cells. As a negative control, competent MG1655 and JW2019-1 cells were transformed with distilled water. As a positive control, pCR™4-TOPO® without the *wbbL* insertion was transformed into competent MG1655 or JW2019-1 cells. Following a one hour incubation at 37ºC and 200 RPM, 100µL of undiluted, transformed cells were plated on LB plates containing appropriate antibiotics (50µg/mL ampicillin for MG1655 cells; 50µg/mL ampicillin and 50µg/mL kanamycin for JW2019-1 cells) (9). Positive and negative controls were plated on separate LB antibiotic plates. Competent cells were plated on LB plates without antibiotics, to ensure the viability of the cells. The plates were incubated at 37°C overnight.

T4 bacteriophage stab assay. The stab assay was performed as described by Beskrovnaya *et al.* (8). MG1655, DFB1655 L9, and JW2019-1, as well as MG1655 and JW2019-1 transformed with pCR™4-TOPO®-*wbbL+*, were tested for susceptibility to T4 bacteriophage. Bacteria were spread-plated onto LB agar containing the appropriate antibiotics and 1mM $CaCl_2$. The agar was stabbed in the center of the plate with T4 bacteriophage stock using the back of a sterile cotton swab. The plates were then incubated for 16-20 hours at 37ºC. The presence of zones of lysis on the bacterial lawn confirmed bacterial susceptibility to T4 bacteriophage.

RESULTS

MG1655, DFB1655 L9, JW2019-1, and WG1 strain *wbbL* genotypes were confirmed using PCR and gel electrophoresis. Before performing our stab assay, we wanted to confirm the *wbbL* genotype of our strains using PCR amplification**.** A band was observed at 1994 bp

for MG1655 and JW2019-1 strains. This was expected and represents the *wbbL* gene containing the IS5 insertion (Fig. 2A). DFB1655 L9 showed a band at 1994 bp and a smaller band at 799 bp which is indicative of the complementation of the *wbbL* gene on a plasmid vector (Fig. 2A). Since the entire DFB1655 L9 genome was subject to PCR, additional bands were observed around 1600 bp, 2500 bp, and 3000 bp, potentially indicating non-specific amplicons. (Fig. 2A). For WG1 which only contains an intact *wbbL* gene, we observed a band at 799 bp, as expected (Fig. 2B). Finally, a single band at 799 bp was observed for pCR™4-TOPO®-*wbbL*+, as expected (Fig. 2). pUC19 plasmid DNA was used as a positive control (PC), and a band was observed at 189 bp which was expected (Fig. 2). Lastly, a no template control (NTC) showed very faint bands around 200 bp which may be attributed to primer dimerization (Fig. 2).

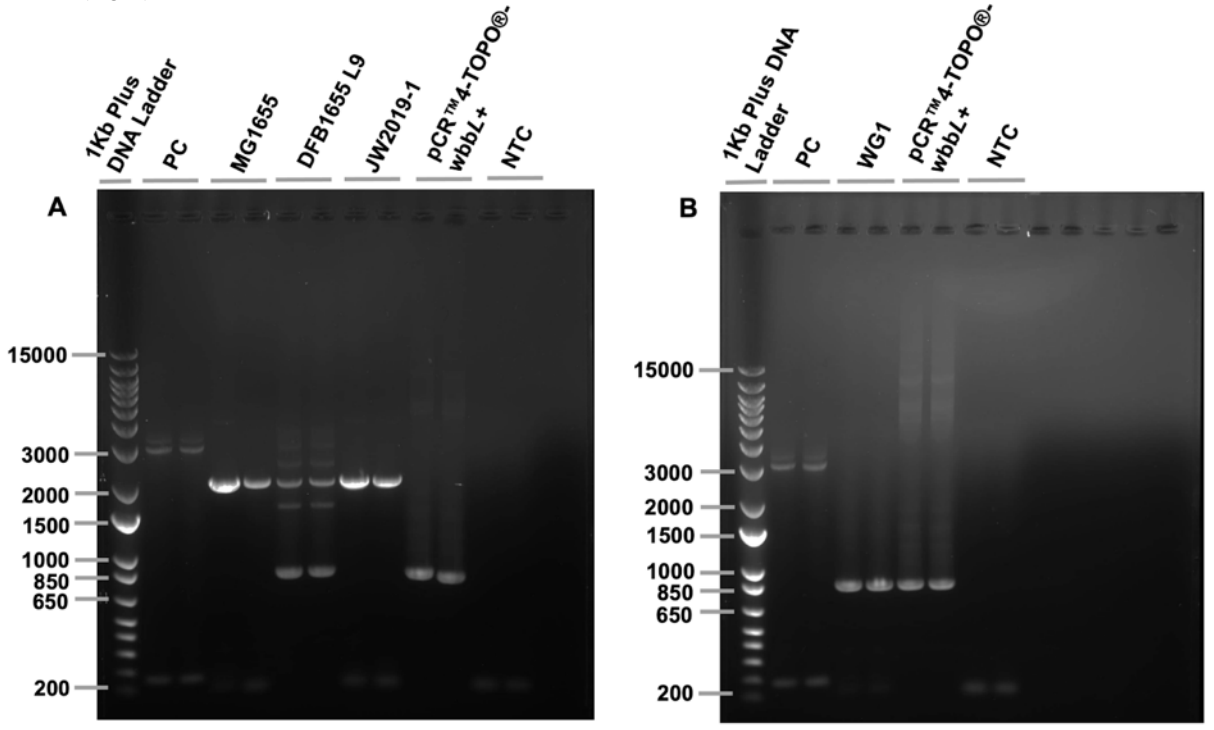

FIG. 2 Confirmation of *E. coli* strain *wbbL* genotypes via PCR and gel electrophoresis. PCR products were run with a 1 Kb Plus DNA ladder (Thermo Fisher Scientific) on a 1% agarose gel. pUC19 was used as a positive control (PC) with an expected band size of 189 bp, while distilled water was used as a no template control (NTC). (A) PCR was used to amplify *wbbL* from strains MG1655 (lanes 4-5), DFB1655 L9 (lanes 6-7), and JW2019-1 (lanes 8-9), as well as the pCR™ 4-TOPO®-*wbbL*+ (lanes 10-11). A band size of 1994 bp was expected for MG1655, DFB1655 L9, and JW2019-1, while a band of 799 bp was expected for DFB1655 L9 and the pCR™ 4-TOPO®-*wbbL*+. (B) PCR was used to amplify *wbbL* from strain WG1 (lanes 4-5) with an expected band size of 799 bp. PC denotes pUC19 positive control and NTC denotes no template control.

JW2019-1 transformed with *wbbL* to generate a *wbbI* knockout remains susceptible to T4 bacteriophage. After successful confirmation of the *wbbL* genotype of our strains, we next performed a stab assay on wild type MG1655, DFB1655 L9, and JW2019-1. To determine the resistance to T4 bacteriophage for each *E. coli* K-12 strain, the zones of lysis were visualized (Fig. 3). For MG1655, a zone of lysis was observed, indicating that MG1655 was susceptible to T4 bacteriophage (Fig. 3B). MG1655 served as our negative control, as it contains the IS5 insertion in the *wbbL* gene and therefore does not express the O16 antigen. For DFB1655 L9, no zone of lysis was observed, indicating that DFB1655 L9 was resistant to T4 bacteriophage (Fig. 3A). DFB1655 L9 served as our positive control, as it contains both the IS5 insertion in the *wbbL* gene and an intact *wbbL* gene on a plasmid, which restores the expression of O16 antigen. For JW2019-1, a zone of lysis was observed, indicating that

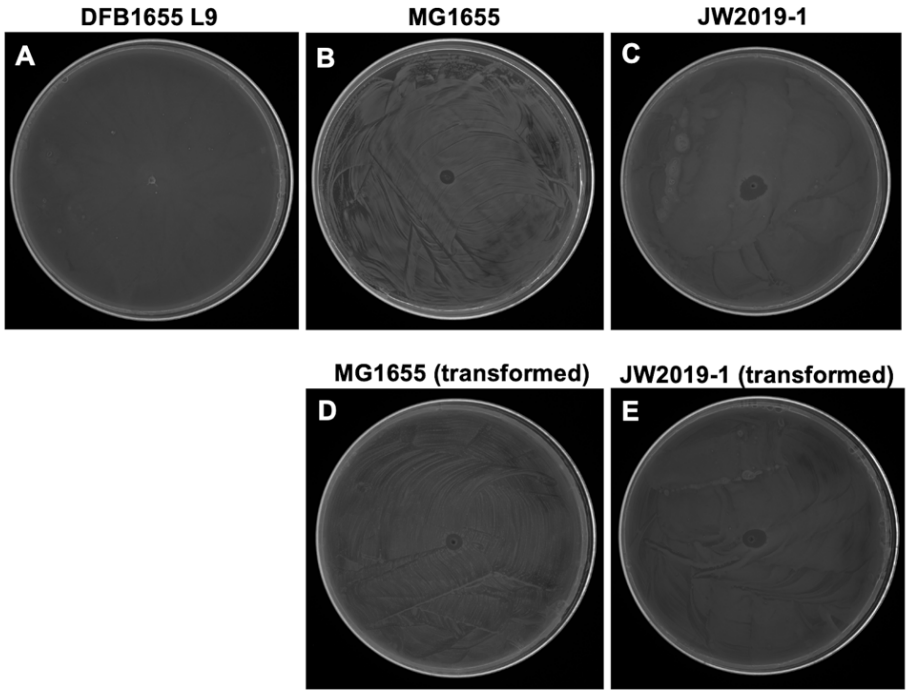

FIG. 3 Stab assay to determine T4 phage resistance in all *E. coli* K12 strains. Zones of lysis are shown in the center of each plate for (A) DFB1655 L9, (B) MG1655, (C) JW2019-1, (D) MG1655 transformed with pCR™4-TOPO®-*wbbL*+, and (E) JW2019-1 transformed with pCR™4-TOPO®-*wbbL*+. Cells were spread-plated for each strain onto LB agar plates containing the appropriate antibiotics and 1mM $CaCl_2$, and then stabbed with concentrated T4 bacteriophage using the back of sterile cotton swab. Plates were incubated for 16-20 hours at 37ºC.

JW2019-1 was susceptible to T4 bacteriophage (Fig. 3C). This result is expected due to the *wbbI* knockout in JW2019-1. Further, this result is consistent with that observed for MG1655, as both strains do not express the O16 antigen as a result of the IS5 insertion into *wbbL.*

In order to generate a strain that had a bona fide *wbbI* knockout and to determine the effect of a *wbbI* knockout on resistance to T4 bacteriophage, JW2019-1 was transformed with pCR™4-TOPO®-*wbbL*+. Henceforth, JW2019-1 transformed with pCR™4-TOPO®-*wbbL*+ will be referred to as *wbbI* knockout. The stab assay results for the *wbbI* knockout demonstrated a zone of lysis, as observed for wild type JW2019-1 (Fig. 3E). This indicates that the *wbbI* knockout remained susceptible to T4 bacteriophage. MG1655 was transformed with pCR™4-TOPO®-*wbbL*+ in parallel to serve as a control. The stab assay result for transformed MG1655 demonstrated a zone of lysis similar to wild type MG1655 (Fig. 3D), indicating that transformed MG1655 remained susceptible to T4 bacteriophage. This result was surprising, as we expected transformed MG1655 to demonstrate the same T4 bacteriophage resistance phenotype as DFB1655 L9.

DISCUSSION

In this study, we observed that the *wbbI* knockout remained susceptible to T4 bacteriophage, as indicated by our stab assay results and the presence of zones of lysis. These results were replicated in an additional stab assay (Fig. S1). WbbI catalyzes the addition of the terminal sugar moiety, D-galactofuranose, to D-glucose of the growing O16 antigen (12). Therefore, when *wbbI* is knocked out in *E. coli*, it results in a truncation of the O16 antigen (12). As previous studies have demonstrated that *E. coli* K-12 strains are resistant to T4 bacteriophage when the intact O16 antigen is expressed (4, 5), we hypothesized that the absence of D-galactofuranose from the O16 antigen would result in decreased resistance to T4 bacteriophage. However, our controls failed which rendered our stab assay results inconclusive. Therefore, we were unable to determine the effect of a *wbbI* knockout on T4 bacteriophage resistance.

Prior to performing the stab assay to determine the effect of a *wbbI* knockout on T4 bacteriophage resistance in *E. coli* K-12, we aimed to confirm the *wbbL* genotypes for our control strains using PCR amplification and gel electrophoresis (Fig. 2). As expected, we observed a single band around 2000 bp for both MG1655 and JW2019-1, which is consistent with expected band size of 1994 bp denoting *wbbL* containing the IS5 insertion (Fig. 2A). For

DFB1655 L9, we again observed the expected band around 2000 bp, and a second band around 850 bp, which is consistent with the expected band size of 799 bp denoting *wbbL* restored on a plasmid (Fig. 2A). As these results confirmed the *wbbL* genotypes of our strains, we were able to proceed to the stab assay with confidence.

Prior to testing the effect of a *wbbI* knockout, we performed a stab assay to determine the susceptibility of various *E. coli* K-12 control strains to T4 bacteriophage. Specifically, we utilized the isogenic *E. coli* strains MG1655 and DFB1655 L9 as our controls, as previous studies have illustrated the differences in T4 bacteriophage resistance between these strains (3-5). MG1655 served as our negative control because it does not express the full O16 antigen due to the IS5 insertion in *wbbL*. The results of the stab assay with MG1655 showed a zone of lysis which indicated that MG1655 was susceptible to T4 bacteriophage (Fig. 3B). DFB1655 L9 served as our positive control, as it expresses the full O16 antigen due to the restoration of *wbbL* on a plasmid. The results of the stab assay with DFB1655 L9 demonstrated no zone of lysis, which suggested that DFB1655 L9 was resistant to T4 bacteriophage (Fig. 3A). Together, these two results confirmed the findings of Dimou *et al.* (4) and Morgan *et al.* (5). Additionally, we used the wild type *E. coli* strain JW2019-1 as a control to demonstrate the baseline susceptibility of this strain to T4 bacteriophage. The results of the stab assay for JW2019-1 demonstrated a zone of lysis, indicating the JW2019-1 was susceptible to T4 bacteriophage (Fig. 3C). This was in accordance with our expectations, as JW2019-1 does not express the full O16 antigen due to the IS5 insertion in *wbbL*.

In order to generate a bona fide *wbbI* knockout, we transformed JW2019-1 with the pCR™4-TOPO®-*wbbL*+ vector to restore the expression of *wbbL*. The results of the stab assay with the *wbbI* knockout showed a zone of lysis like that of wild type JW2019-1 (Fig. 3E). In order to verify that the stab assay results observed for the *wbbI* knockout were due to the reinsertion of *wbbL*, we transformed MG1655 in parallel with *wbbL* with the aim of restoring the expression of the O16 antigen. Based on the observation that DFB1655 L9 demonstrates resistance to T4 bacteriophage due to the restoration of *wbbL* on a plasmid (3-5), we expected that MG1655 transformed with *wbbL* would demonstrate no zone of lysis, as the transformed MG1655 strain would theoretically be identical to DFB1655 L9. Contrary to this expectation, the stab assay results for MG1655 transformed *wbbL*, demonstrated a zone of lysis similar in size to that of wild type MG1655 (Fig. 3D). This suggests that MG1655 transformed with *wbbL* did not confer resistance to T4 bacteriophage. A possible explanation of this result could be that the transformed *wbbL* gene is not being expressed, as we did not confirm the expression of the pCR™4-TOPO®-*wbbL*+ vector following transformation into MG1655. If this was the case, this would result in no expression of the truncated O16 antigen on the surface of MG1655. Consequently, transformed MG1655 would remain susceptible to T4 bacteriophage as there is still no steric hindrance conferred by the O16 antigen preventing T4 bacteriophage tails from attaching to OmpC. Therefore, we cannot draw any conclusions about the T4 bacteriophage resistance phenotype for the *wbbI* knockout based on the stab assay results.

Limitations A limitation of this study is that due to the qualitative nature of our stab assay, we were unable to quantify the differences in T4 bacteriophage resistance between the different strains. Another limitation associated with this study is that, due to time constraints, we were unable to perform a plaque assay to confirm the T4 bacteriophage titer. As a result, the titer that produced the stab assay results is unknown. A limitation associated with our experimental design is that our method of verifying O16 antigen expression relied on MG1655 exhibiting the same phenotype as DFB1655 L9 upon transformation with pCR™4-TOPO®-*wbbL*+. However, based on our stab assay, the transformed MG1655 cells did not exhibit the expected result, meaning that our control to confirm O16 antigen expression failed. This may be due to the *wbbL* gene not being expressed in transformed MG1655 cells. Thus, we cannot be certain if *wbbL,* and therefore the truncated O16 antigen, was expressed in the *wbbI* knockout. Since we did not have another method of confirming O16 antigen expression, we cannot draw any conclusions from the stab assay results with transformed bacteria.

Conclusions Our study investigated the effect of a *wbbI* knockout on T4 bacteriophage resistance. Although the *wbbI* knockout displayed susceptibility to T4 bacteriophage on the stab assay, we were unable to draw any conclusions about the effect of *wbbI* on T4 bacteriophage resistance as our transformed MG1655 control failed to exhibit the expected phenotype. As such, the mechanism of resistance to T4 bacteriophage in *E. coli* K-12 remains unclear.

Future Directions Future work should consider incorporating methods into the protocol to ensure the *wbbL* gene is being expressed in the cell after transformation. Specifically, this could be achieved by utilizing a GFP-tag or performing a quantitative reverse transcription PCR (RT-qPCR) analysis. Further, future studies should use a silver staining assay to verify that the truncated O16 antigen is indeed being expressed on the surface of JW2019-1 transformed with *wbbL*. In order to examine whether T4 tails are adsorbing to the cell surface, transmission electron microscopy techniques could be used. Additionally, while this study utilized a qualitative assay to test resistance to T4 bacteriophage, future studies should consider performing a quantitative assay to test bacteriophage resistance. For instance, an adsorption assay may be used to quantitatively measure the ability of T4 bacteriophage to adsorb to *E. coli* strains. Lastly, as an alternative to transforming *wbbL* into JW2019-1, future studies should consider designing an antisense RNA to silence *wbbI* in DFB1655 L9. These directions could help provide a clearer understanding of the effects of a *wbbI* knockout on resistance to T4 bacteriophage.

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CONTRIBUTIONS

All individuals of the team were integral to the planning and execution of this project, as well as writing and editing this manuscript. S.A., C.E., P.G., and C.W. were involved in the planning and performing of PCR, gel electrophoresis, and stab assays. Additionally, P.G. was the primary communicator with our lab colleagues, Eleanor Chen, Camila Quintana, and Daniel Song. S.A generated Figure 1, and C.W generated Tables 1 and 2, and Figures 2, 3, S1.

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