Volume 7:1-11

The Undergraduate Journal of Experimental Microbiology & Immunology (+Peer Reviewed)

Review: Restoration of O antigen expression in *Escherichia coli* K-12 MG1655 confers bacteriophage resistance

Wee So Kim

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

SUMMARY The objective of this review is to summarize research that has been done on O antigen-mediated bacteriophage resistance in *E. coli* K-12 and to investigate the potential mechanisms of resistance. The loss of O antigen expression in *E. coli* K-12 strains such as MG1655 is due to the disruption of *wbbL* gene with an IS5 element. Insertion of an intact *wbbL* gene from O antigen expressing strain of *E. coli* WG1 into the chromosome of *E. coli* MG1655 restored O antigen expression. The resulting strain, DFB1655 L9, is resistant to bacteriophages P1, T4, and T7. Bacteriophage absorption was measured using qPCR and a double agar overlay plaque assay, which showed a greater number of bacteriophages in the supernatant of an O antigen producing *E. coli* strain. Visualization of T4 bacteriophage and host interactions using negative stain electron microscopy showed a reduced amount of bacteriophage on strains expressing O antigen. These data suggest that bacteriophage absorption may be inhibited by O antigen. This review concludes by considering several steps in the bacteriophage life cycle at which O antigen may inhibit T4 infection.

INTRODUCTION

Bacteriophages are viruses found ubiquitously in the environment that infect bacteria in order to replicate, acting as obligate parasites. Bacteriophage T4 is a T-even bacteriophage capable of infecting and inducing lysis in the extensively studied Gram-negative bacterium, *Escherichia coli* (1-3). Bacteriophage T4 or phage T4 is a relatively large bacteriophage with an icosahedral head that carries a 169 kb double-stranded DNA genome (4). The head is attached to a hollow tube connected to short and long tail fibers, which mediate host recognition and attachment (3, 4). Viral absorption consists of the following steps: initial bacteriophage contacts, reversible binding, and irreversible attachment (3). The phage binds to receptors reversibly with the long tail fibers, then binds irreversibly with the short tail fibers (1). The overall life cycle of T4 bacteriophage is illustrated in Figure 1.

As the natural prey of bacteriophage T4, *E. coli* are Gram-negative bacteria with an asymmetric outer membrane predominantly composed of lipopolysaccharide (LPS) molecules in the outer leaflet. LPS consists of lipid A, which serves as an anchor for non-repeating oligosaccharide that form the core of LPS. A repeating polysaccharide consisting of 3 to 8 sugar groups, called the O-polysaccharides or O antigen, is attached to the core (3, 5, 6). *E. coli* serotypes are determined by the type of polysaccharide antigens on their cell membrane including the O antigen (3). LPS has been shown to be one of the surface receptors for bacteriophage adsorption of T4 phages (3, 7). The *E. coli* osmoregulator, OmpC, can also serve as a receptor for T4 (3). The absorption of T4 can also be supported when either LPS or OmpC is available (2). Previous studies have found that mutations preventing OmpC expression or the addition of specific sugar residues in LPS make *E. coli* more resistant to T4 bacteriophage infection (3, 8). Another T-even bacteriophage, T7, can also infect Gram-

Published Online: September 2021

Citation: Wee So Kim. 2021. Review: Restoration of O antigen expression in Escherichia coli K-12 MG1655 confers bacteriophage resistance. UJEMI+ 7:1-11

Editor: Daniela Morales, Stefanie Sternagel and Brianne Newman, University of British Columbia

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

negative bacteria using specific cell surface receptors (16). Same as T4, the core region of LPS is identified as the primary receptor for T7 phage (16). Another surface molecule utilized by T7 is OmpA/OmpR (16).

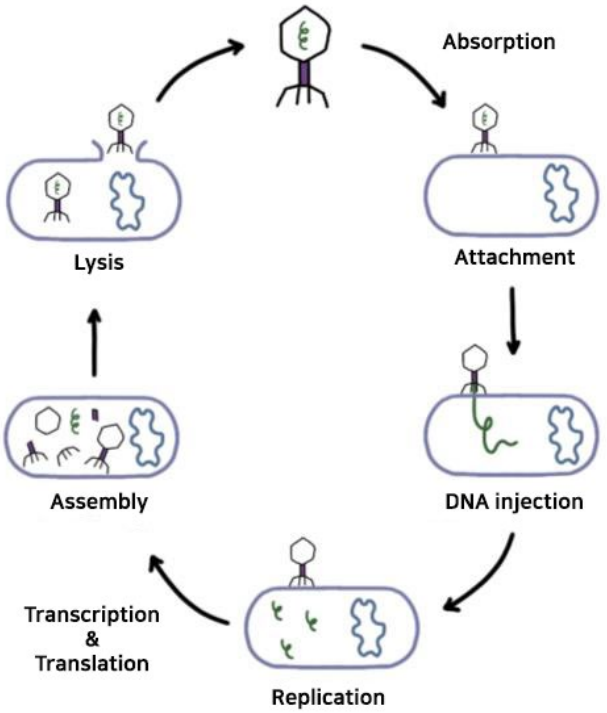


FIG. 1 Bacteriophage T4 life cycle. Phage infection is initiated by the interaction between bacteriophage fiber tails and specific receptors on host cell surfaces (Attachment). Once the interaction is irreversible, the bacteriophage injects its genome into the host (DNA injection). The viral genome rapidly replicates using host cell machinery and produces viral protein (Replication). By assembling viral parts, new bacteriophage are formed and released from the host (Assembly and Lysis). Adapted from Salmond and Fineran (4). Figure created in ProCreate.

E. coli K-12 has been used as a model organism in laboratory settings for decades, which has given rise to an accumulation of mutations that have resulted in the loss of its ability to synthesize the outer membrane component, O antigen (9, 10). To investigate the role of O antigen in bacteriophage infections, Browning *et al*. created an isogenic strain of K-12 MG1655 by re-introducing the gene cluster responsible for O antigen synthesis into its genome (11). The resulting isogenic strain, DFB1655 L9, is able to synthesize O antigen and resist infection by a specific bacteriophage, P1 (11). Both bacteriophage T4 and P1 use LPS as their host receptor (13). This observation of phage resistance in bacteriophage P1 lead to of an extensive investigation on the role of O antigen during phage infection in bacteriophage T4 and T7 (14-20).

This review describes the literature on T4 resistance of engineered *E. coli* K-12 strain DFB1655 L9, which is capable of expressing O antigen. This review will outline the 1) O antigen synthesis pathway, 2) O antigen synthesis restoration in strain DFB1655 L9, 3) O antigen mediated T4 resistance in strain DFB1655 L9, 4) T4 resistance by preventing bacteriophage adsorption, and 5) potential models of T4 resistance. Finally, this review discusses how bacteriophage resistance in *E. coli* K-12 could contribute to the development of possible therapeutics using bacteriophage.

O antigen biosynthesis in *E. coli* K-12 requires *wbbL*. *E. coli* K-12 has been passaged in lab media repeatedly over decades resulting in a loss of genetic characteristics including the F plasmid, bacteriophage λ, and expression of cell surface structures including the O antigen (9, 10). The loss of O antigen expression is a result of the disruption of *wbbL* gene by a small transposable insertion sequence (IS), IS5, also called the *rfb*-50 mutation (Fig. 2) (11). The gene cluster for O antigen is called *rfb* gene cluster (12). As shown in Figure 2, *wbbL* is one of the genes in the O antigen biosynthesis operon which also consists of *wbbK, wbbJ* and *wbbI* (12). These Wbb proteins are responsible for catalyzing covalent linkages between the sugar building blocks that make up O antigen (Fig. 3). O antigen synthesis is initiated by the WbbL protein, a rhamnose transferase that adds L-rhamnose to the D-N-acetylglucosamine molecule anchored in the cytoplasmic side of the inner membrane using undecaprenyl pyrophosphate (Und-PP) (Fig. 3). Once L-rhamnose is bound to the growing O antigen

residue, WbbK adds D-glucose. Then WbbJ adds O-acetyl to L-rhamnose, and WbbI adds D-galactofuranose. The O antigen is then translocated across the inner membrane by the Wzx translocase (6). A complete O antigen molecule is synthesized in the periplasm through the polymerization of repeating units until it achieves the preferred modal chain length (6). Once a complete O antigen molecule is ready, the entire unit is ligated to a lipid-A core and translocated to the outer membrane (6). Since translocase Wzy can also translocate an incomplete repeating unit to lipid A-core, a truncated version of O16 with an incomplete repeating unit is possibly presented on the cell surface. This process is demonstrated with O7, which has the same first sugar residue as O16, D-N-acetylglucosamine (Fig. 3) (6).

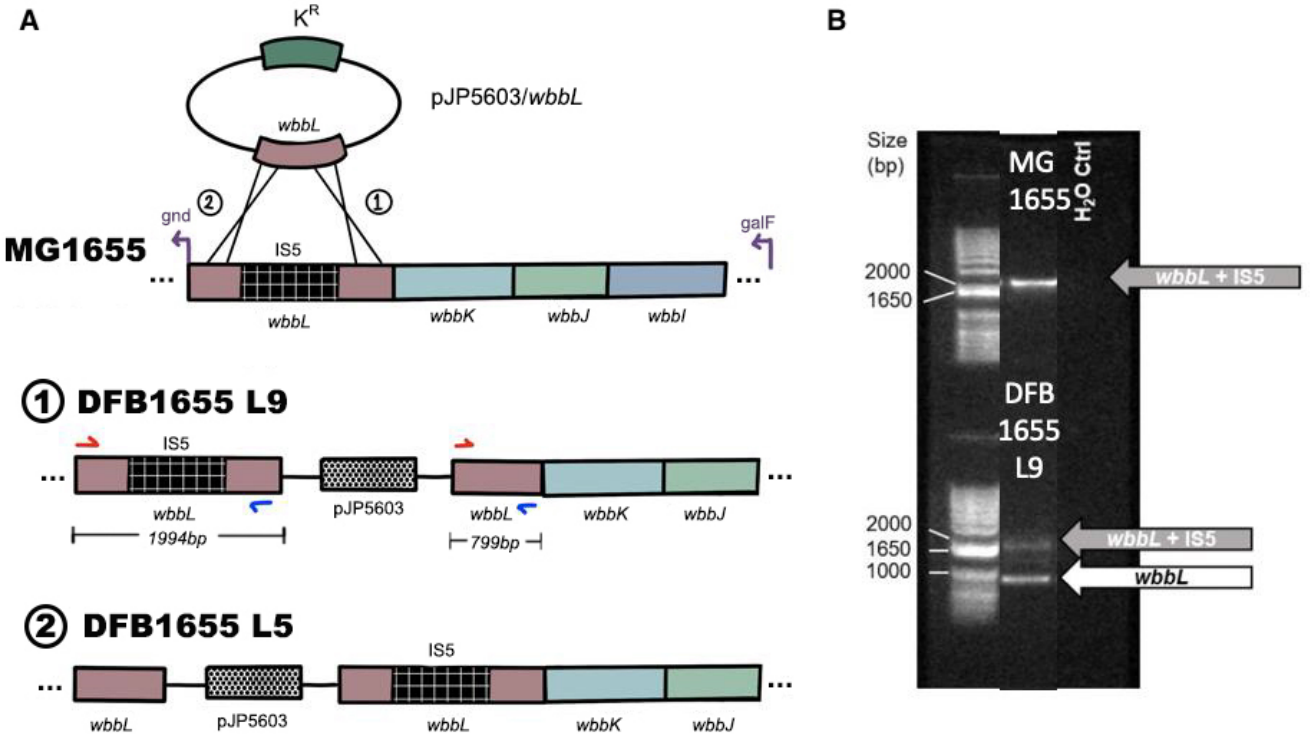


FIG. 2 Restoration of O antigen using plasmid complementation to insert wild-type *wbbL* gene into the host chromosome. (A) The conjugative suicide vector pJP5603 with the *wbbL* gene is integrated into the MG1655 chromosome to regenerate the *rfb* gene cluster. The insertion of *wbbL* upstream of IS5 resulted in strain, DFB1655 L9 (shown as cross 1). The integration of *wbbL* downstream of IS5 resulted in the DFB1655 L5 strain (cross 2) (9). The forward and backward primers used to detect the insertion of WT *wbbL* are indicated as yellow and green arrows in the figure. Adapted from Browning *et al*. (9) and created in ProCreate. The genes drawn here are not to scale. (B) Colony gradient PCR results of MG1655 and DFB1655 L9. In DFB1655 L9, the amplicon in size of 800 bp was observed, which represents the *wbbL* gene (white arrow). The amplicon in size of 2000 bp was observed in both strains, which indicates the disrupted *wbbL* gene with the 1200 bp IS5 element (grey arrows). A negative control (H_2O) was included on the right top of the gel. Reproduced from Chiu *et al*. (10).

Restoration of O antigen synthesis in *E. coli* strain MG1655. To understand the role of O antigen in *E. coli*, Browning *et al*. conducted a study to restore O antigen expression by introducing a functional copy of the *wbbL* gene into the genetically well-characterized *E. coli* strain MG1655. To confirm that the *rfb*-50 mutation is the only factor responsible for O antigen synthesis in *E. coli*, Browning *et al*. cloned wild-type *wbbL* from *E. coli* strain WG1 into the pET20b vector and transformed this plasmid into MG1655 in a gain of function experiment (Fig. 2) (11, 12). The total cellular protein and LPS expressed in MG1655 transformed with a negative control vector (pET20b/*wbbL* with a single base pair deletion) or WbbL expressing vector (pET20b/*wbbL)* were compared (11). Only the pET20b/*wbbL* expressing strain synthesized O antigen when measured using a silver-stained SDS-PAGE (11). Browning *et al.* confirmed that MG1655 was incapable of producing O antigen synthesis due to the disruption of *wbbL* (11).

The regeneration of *rfb* cluster was achieved by vector complementation of *wbbL* into the MG1655 genome through chromosomal insertion (Fig. 2) (11). The *rfb* cluster was structurally regenerated when the plasmid was integrated upstream of the IS5 element, which generated DFB1655 L9 strain (Fig. 2) (11). However, when *wbbL* was inserted downstream of the IS5 element, both undisrupted *wbbL* gene and disrupted *wbbL* exist together and the *rfb* gene cluster is not regenerated with the remaining IS5 element, which generated DFB1655 L5 strain (Fig. 2) (11). Using silver-stained SDS-PAGE of LPS, the expression of O antigen on LPS in both strains was demonstrated, however, the amount was significantly greater in DFB1655 L9 than DFB1655 L5, where DFB1655 L5 exhibited wild-type (MG1655) levels of LPS (11). These newly produced strains are isogenic to MG1655 (11). Interestingly, DFB1655 L5 behaved similar to MG1655 in terms of bacteriophage resistance, suggesting that perhaps the density of O antigen on the cell surface may play a role in bacteriophage resistance. A recent study has shown that lysogenization of O antigen producing *E. coli* is due to the loss or reduced amount of O antigen (24). Thus, developing a separate isogenic strain with a non-native, inducible promoter could potentially be used to induce increased levels of O antigen by adding activators to enhance the interaction between the promotor and RNA polymerase. The altered level of O antigen synthesis could help determine whether resistance is density dependent.

Besides O antigen production, there was no difference in cell growth, cell viability, outer membrane composition and OmpC production observed between DFB1655 L9 and MG1655 (11). Browning *et al*. observed that DFB1655 L9 is resistant to bacteriophage P1, exhibits decreased transformation efficiency, more pathogenic to *Caenorhabditis elegans* by resisting mechanical force, and exhibits reduced biofilm production compared to MG1655 and DFB1655 L5 (11).

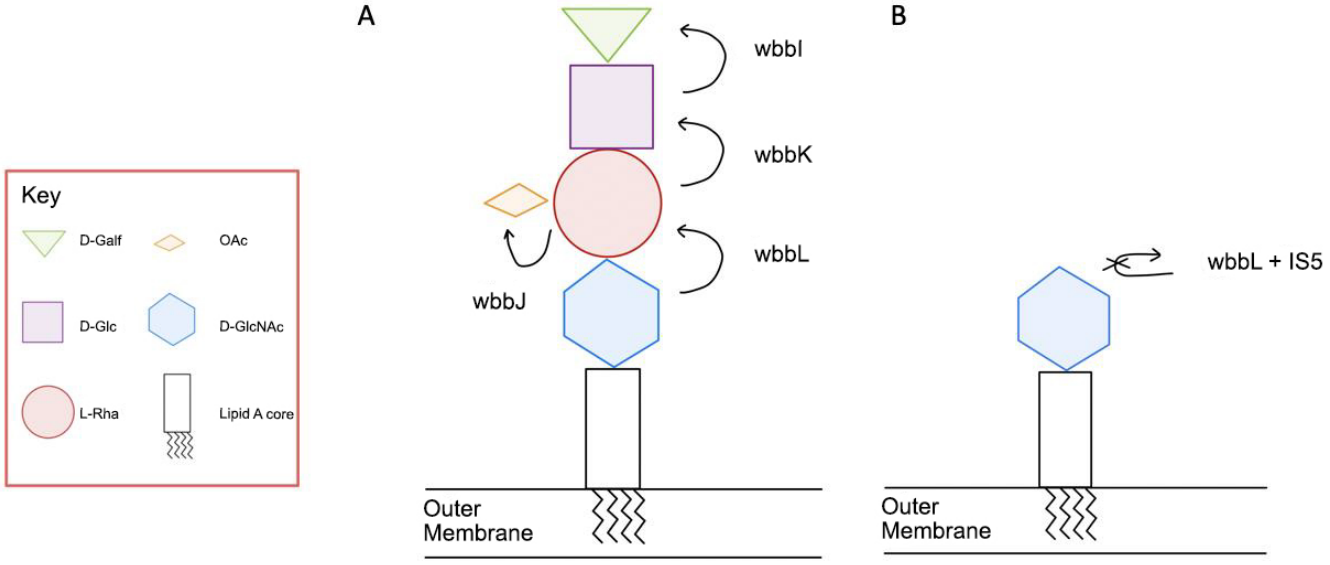


FIG. 3 O16 antigen biosynthesis pathway of *E. coli* K-12. (A) A complete O16 antigen presented on the *E. coli* K-12 cell surface with *wbbL* expression. (B) A truncated O16 antigen in the absence of WbbL. Abbreviations: Galf, galactofuranose; Glc, glucose; GlcNAc, N-acetylglucosamine; OAc, O-acetyl; Rha, rhamnose. Reproduced from Hong *et al*. (6) and created in Goodnotes.

Restored O antigen production in *E. coli* renders them resistant to bacteriophages. Given that pathogenic *E. coli* strain O157 H7, which expresses O antigen, is resistant to bacteriophage P1, Browning *et al*. hypothesized that strain DFB1655 L9 may show similar resistance to P1 (11, 13). A cross-streak assay was conducted where each bacterial strain was streaked perpendicular across a line of P1 lysate on petri plates containing Luria broth (LB) agar media (11). A zone of clearing was observed when strain MG1655 was cross-streaked with P1, but DFB1655 L9 grew as a solid line when cross-streaked with P1 (11). These results suggest that the presence of a functional *wbbL* gene in strain DFB1655 L9 renders *E. coli* resistant to bacteriophage P1 infection (11). To follow up on this finding, the student team of Chiu *et al*. hypothesized that DFB1655 L9 may be resistant to other types of

bacteriophage such as T4, which shares the same host surface receptor LPS (14). Chiu *et al*. used a double agar overlay plaque assay to measure T4 resistance of MG1655 and DFB1655 L9 (14). In this assay, each strain was mixed with T4 in agar to allow bacteriophage-bacterium interactions. The mixture was then poured onto petri plates and incubated (14). The number of plaques formed in the bacterial lawn were counted the next day (14). There were no plaques observed from DFB1655 L9 plates at all multiplicities of infection (MOI) tested. In contrast, complete lysis or large numbers of plaques were observed on the MG1655 plates indicative of strain sensitivity to T4 (14). Taken together, the data suggests that O antigen expression in strain DFB1655 L9 confers resistance to T4 infection (14). The results were recapitulated by several research teams (15-20). The team of Lee *et al*. hypothesized that T7 bacteriophage infection of DFB1655 L9 may yield a similar result (16). T7 infectivity in DFB1655 L9 and MG1655 was tested using the double agar overlay plaque assay (16). The result showed that DFB1655 L9 is also resistant to T7, whereas MG1655 was susceptible (16). These observations indicate the presence of O antigen renders *E. coli* strain K-12 resistant to multiple bacteriophages including P1, T4, and T7 (11, 14-20). However, the mechanism of O antigen-mediated bacteriophage resistance in *E. coli* remained to be elucidated.

Bacteriophage T4 infection of MG1655 is not inhibited by secreted factors from DFB1655 L9. The observation that the restoration of an intact copy of *wbbL* was sufficient to confer resistance to bacteriophage infection suggests that a component of O antigen may interact with the bacteriophage. One model that may explain the DFB1655 L9 bacteriophage resistant phenotype is the soluble factors such as a component of O antigen that may be released from the bacterial surface into the culture supernatant. Bacteriophage may bind to this secreted moiety thereby inhibit bacteriophage infection via chemical interaction that leads to inactivation (14). Since the presence of O antigen in DFB1655 L9 renders T4 resistance, it is possible that T4 may interact with free O antigen in the environment. To test this, Chiu *et al*. incubated T4 with *E. coli* culture supernatant overnight prior to infecting MG1655 at MOI of 1 (14). The supernatants are expected to contain secreted factors from each cell. Using a double agar overlay plaque assay, no difference in plaque formation was observed from T4 incubated with MG1655 or DFB1655 L9 soluble factors (14). This suggests that the soluble factors released from *E. coli* strain DFB1655 L9 into the culture supernatant do not restrict bacteriophage infection. However, in this experiment, the composition of released DFB1655 L9 soluble factors in the supernatant was not defined. Therefore, by incubating T4 with purified O antigen may provide further insight into the interaction between T4 bacteriophage and O antigen.

O antigen may inhibit bacteriophage adsorption to mediate resistance. Chiu *et al*. proposed that O antigen may hinder binding of bacteriophage receptor on *E. coli* LPS or OmpC. Wachtel *et al*. followed up on this idea by proposing that O antigen may be inhibiting the adsorption of bacteriophage T4 to the host (13, 15, 21). To test this, Wachtel *et al*. used quantitative polymerase chain reaction (qPCR) to quantify the amount of T4 *gp23* gene in the supernatant of MG1655 or DFB1655 L9 after incubation with phage T4 (Fig. 4A) (15). The experimenters predicted that if O antigen prevented T4 adsorption then more *gp23* would be measured in the culture supernatants (15). In their assay, *E. coli* cells were incubated with bacteriophage T4 for 5 minutes to allow viral adsorption but not bacteriophage replication and host cell lysis (Fig. 4A) (15). After centrifugation, chloroform was added to the collected supernatant to prevent viral replication and disrupt remaining cells (Fig. 4A) (15). qPCR was then used to quantitatively detect T4 *gp23* (15). DFB1655 L9 showed lower Cq values than MG1655 at all bacteriophage concentration tested, indicating that there was more T4 *gp23* in the DFB1655 L9 supernatant (Fig. 4B, C) (15). There was an average of 4.9×10^6 more copies of bacteriophage genome detected in the supernatant of DFB1655 L9 than MG1655 at 10^{-3} MOI, which implies that bacteriophage adsorption is inhibited in DFB1655 L9 (15). However, the major limitation of qPCR is its ability to quantify the number of "active" bacteriophages in a sample. This assay could be detecting all nucleic acids in the sample, which may include nonfunctional bacteriophages or any free bacteriophage genome that is released into the environment prior to absorption. Thus, measuring T4 bacteriophage using

qPCR may result in an over-estimation that may not reflect the exact degree of absorption prevention.

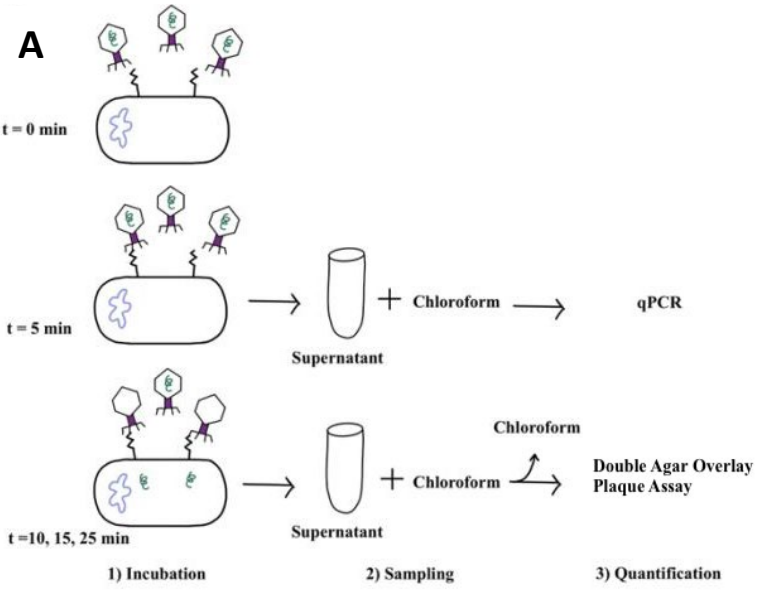
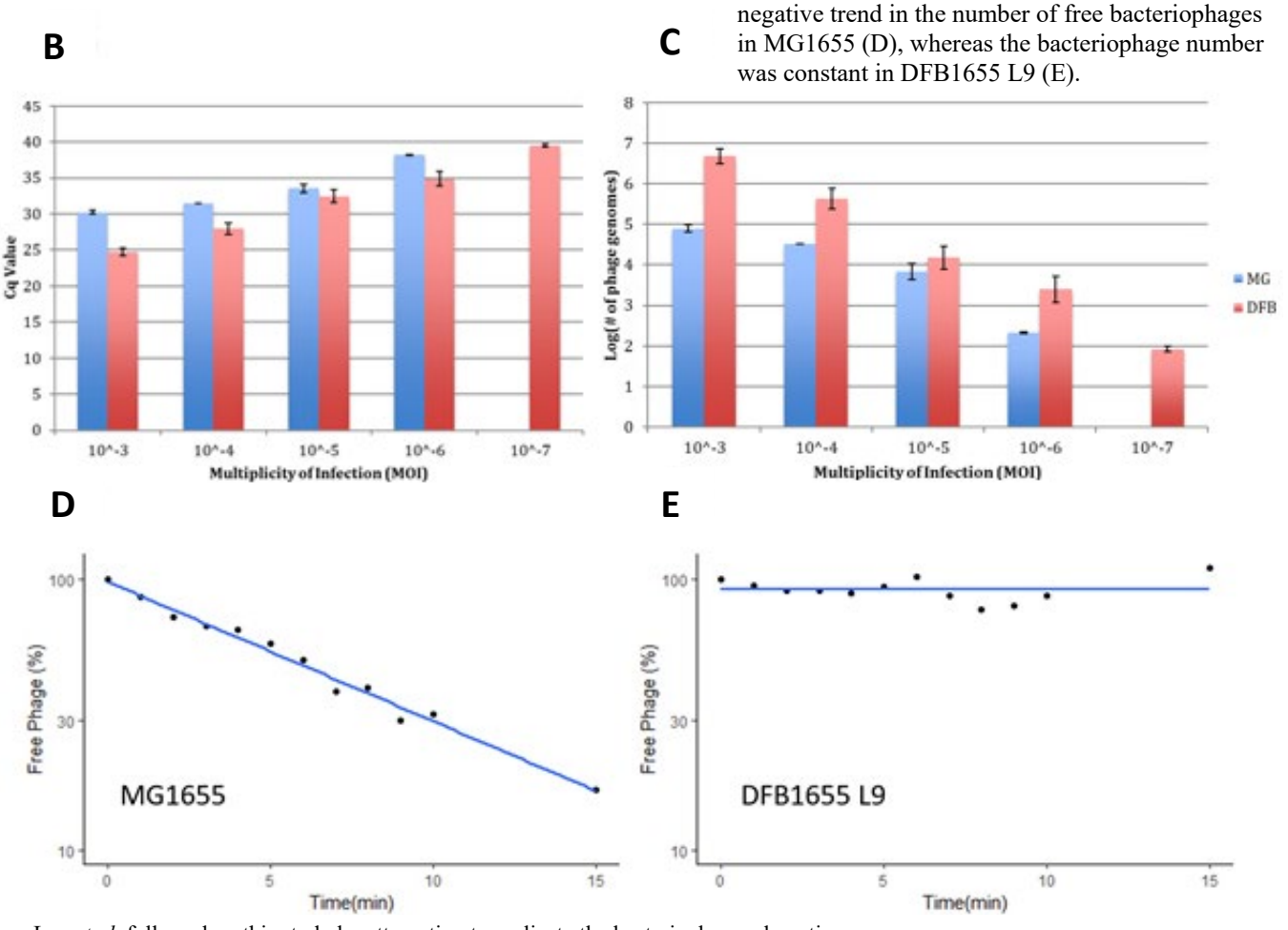


FIG. 4 Quantification of the unbound T4 bacteriophage particles in the supernatant of *E. coli* K-12 culture using qPCR and a double agar overlay plaque assay. (A) Molecular representation of the two assays show that at time point 0, none of the free bacteriophage particles are bound to the bacteria. After 5 minutes, some bacteriophage particles are bound to the receptors on bacterial outer membrane. Remaining free bacteriophage gradually bind to the host receptor over the next 10 to 25 minutes, and some unbound particles are remained in the supernatant. The supernatant of the cultures was then collected following centrifugation and chloroform was added. For quantifying the number of bacteriophages in the supernatant, Wachtel *et al*. (15) used qPCR to measure the levels of T4 *gp23*. Here, MG1655 is represented as blue bars, and DFB1655L9 as red bars. The Cq values and number of bacteriophages calculated are shown in (B) and (C). In contrast, Dimou *et al*. (17) used double agar overlay plaque assay and observed a negative trend in the number of free bacteriophages in MG1655 (D), whereas the bacteriophage number was constant in DFB1655 L9 (E).



Lee *et al*. followed up this study by attempting to replicate the bacteriophage adsorption assay. Using a qPCR assay, Lee *et al*. observed no difference between the number of detected T4 *gp23* or T7 *gp10a* in the supernatant of both MG1655 and DFB1655 L9 cultures (15, 16). The testing condition of each group was identical with the same MOI, primers, procedure

being used (15, 16). Lee *et al*. suspected the failure to replicate the same results could have been caused by any deviation in undocumented techniques or reagent composition (16). Thus, another experiment maybe be needed to confirm whether the result could be reproducible by using Wachtel *et al.*'s bacteriophage adsorption assay.

Given the discrepancy between the studies using qPCR to quantify bacteriophage in the culture supernatant, Dimou *et al.* (2019) adapted an adsorption assay using a double agar overlay plaque assay based on the work by Kropinski *et al.* (Fig. 4A) (17, 23). In their assay, Dimou *et al.* incubated T4 with either MG1655 or DFB1655 L9 for 10 minutes and collected the supernatant every minute. Two additional samples at 15 and 25 minutes were also collected. After centrifugation of collected supernatants, chloroform was added to kill bacterial cells and prohibit reversible binding. Chloroform separated out from the solution after 1-2 minutes of cold incubation, then the chloroform-free supernatant was mixed with *E. coli* cells to conduct a double agar overlay plaque assay (Fig. 4A) (17). Any unbound bacteriophage would result in a plaque in the assay. The number of plaques formed would indicated the proportion of unbound bacteriophage in the culture supernatant. The number of plaques in the MG1655 condition decreased overtime as shown in Figure 4D (17). This suggested that more T4 was binding to MG1655 with increased incubation time. In contrast, no changes in the number of plaques was observed in the DFB1655 L9 cultures incubated with T4 as shown in Figure 4E (17). These data suggest that T4 does not adsorb onto DFB1655 L9. In contrast to qPCR, only functional bacteriophages would infect the bacterial cells in a double agar overlay plaque assay. Therefore, a double agar overlay plaque assay can measure bacteriophage adsorption prevention more accurately and provide more meaningful data. Furthermore, Dimou *et al*. calculated the attachment rate constant of MG1655 and DFB1655 L9, which indicates the degree of irreversible attachment of the bacteriophage to the host cell that is required before the bacteriophage can inject its viral genome into the host. The attachment rate constant for DFB1655 L9 was 0 mL/min(cfu), which indicates that irreversible attachment of T4 bacteriophage to DFB1655 L9 was inhibited (17). Taken together, these results strongly suggest that the expression of O antigen in strain DFB1655 L9 prevents T4 adsorption.

Two teams have attempted to examine the physical interaction between T4 and MG1655 or DFB1655 L9 using negative stain electron microscopy (EM) (18, 19). Biparva *et al*. incubated T4 stock at 1.79 x 10^{10} pfu/mL with DFB1655 L9 or MG1655 for 5 minutes (18). The supernatant was removed and the cells were fixed, washed, and stained for EM preparation (18). Biparva *et al*. observed 3-4 bacteriophage per cell in MG1655. In contrast, no bacteriophages were observed bound to DFB1655 L9 (18). Morgan *et al*. followed up on this result by repeating the EM study with a 9-minute incubation prior to processing at a MOI of approximately 10 (19). Morgan *et al*. observed an average of 7-11 bacteriophage per MG1655 cell and 3 bacteriophages per DFB1655 L9 cell (19). This suggests that the number of T4 phages adsorbing into MG1655 is higher than DFB1655 L9. However, it is worth noting that in both studies, the head of bacteriophage T4 appeared to be embedded in the host cell surface and the interaction between bacteriophage tails and host cell surface was not clear (18, 19). Another observation made by the research groups was that DFB1655 L9 cells appeared elongated and darker than MG1655 (18, 19). Biparva *et al*. speculated that this may due to the uptake of EM stain by O antigen (18). However, Morgan *et al*. observed a mixture of morphological phenotypes in the DFB1655 L9 culture, the same elongated cells observed by Biparva *et al*. and regular wild-type-like cells (18, 19). Taken together, these EM studies observed bacteriophage bound at the bacterial surface and suggest that more bacteriophage may bind per cell in strain MG1655 versus DFB1655 L9.

Models of O antigen mediated bacteriophage resistance. As summarized above, several different mechanisms have been proposed to explain how the expression of O antigen could mediate bacteriophage resistance. The model of *E. coli* soluble factors inhibiting T4 infection was tested, but no difference in bacteriophage infectivity was found (14). Here, the soluble factors are not specific for secreted O antigen (14). This may imply that the secreted factors are not giving protection towards the bacteriophage. However, since it was not specific for O antigen, the amount of released O antigen may be not sufficient for making the bacteria protective. Thus, this model has to be re-examined with only O antigen soluble factors at

difference concentration to verify the exact interaction between bacteriophage T4 and DFB1655 L9 (Fig. 5B).

The idea that O antigen may prevent T4 absorption was tested by using qPCR and double agar overlay plaque assays, which shows that there was a greater amount of free unbound T4 bacteriophage in MG1655 culture than DFB1655 L9 (15-17). For example, the number of phages in the supernatant of DFB1655 L9 was 2.24 x 10^5 pfu/mL and 3.6 x 10^4 pfu/mL in MG1655 after 15 minutes of incubation (17). Furthermore, bacteriophage-host interactions were observed using negative stain EM (18, 19). The EM images showed fewer bacteriophage bound to the surface of DFB1655 L9 compared to MG1655 (18, 19). These observations suggest that O antigen may physically block the contact of T4 with the *E. coli* cell surface receptors (Fig. 5C). The early stage nature of the research problem allows several working models to explain how the restoration of an intact copy of *wbbL* in the MG1655 genome results in resistance to bacteriophage. The following summarizes a number of potential models that may explain this phenotype as depicted in Figure 5.

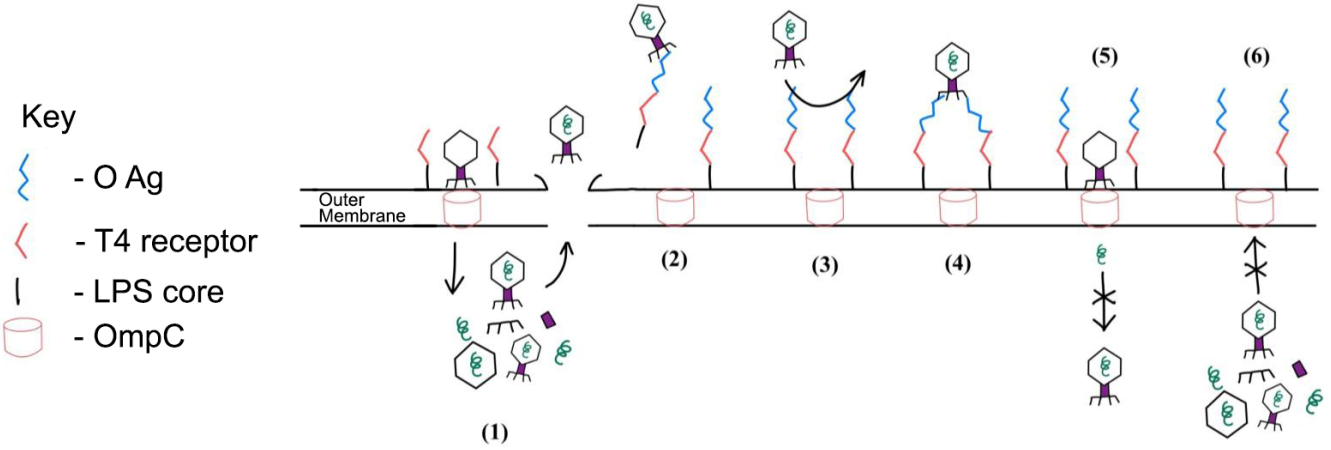


FIG. 5 Potential O antigen mediated T4 resistance models in *E. coli* K-12. (A) When O antigen is absent, T4 bacteriophage can bind and attach to the receptors of *E. coli* K-12 and inject viral DNA into the host. The replication and production of viral components is completed with host machinery and the newly assembled progeny is released from the host through cell lysis. (B) LPS soluble factor with O antigen may interact with free T4 bacteriophages in the environment and prevent bacteriophage binding. (C) O antigen may physically block T4 binding to cell surface receptors. (D) O antigen may trap T4 and prevent interaction with cell surface receptors. (E) O antigen may prevent T4 viral replication. (F) O antigen may prevent T4-induced cell lysis.

O antigen prevents bacteriophage T4 adsorption with physical interference. To avoid bacteriophage infection, the interaction between host cell surface receptor and bacteriophage tails may be inhibited (1, 2). As observed previously, O antigen can serve as a primary receptor during infection for some bacteriophages as well as restricting the access to host cell surface receptors (Fig. 5C) (13, 24, 25). Since O antigen is at the distal end of *E. coli* LPS, T4 bacteriophage will encounter O antigen before the host cell receptors. The long-chain of O antigen may have a protective effect during viral infection by preventing bacteriophage attachment with secondary receptors (25). Thus, by physically blocking bacteriophage particle, the interaction between T4 bacteriophage and host receptors is prevented and may lead to bacteriophage resistance. An additional enzymatic degradation of O antigen is required to access the secondary receptors masked by the O antigen (25). Moreover, the visualization of bacteriophage-bacteria interaction shows that the number of T4 bacteriophage bound to DFB1655 L9 was significantly lower compared to MG1655 (18, 19). The possible explanation of this phenomenon could be electrostatics repulsion since the initial contact of bacteriophage to the host is inhibited. These finding supports the blocking model that suggests bacteriophage approach is physically inhibited by the extensive O antigen layer (Fig. 5C). If the resistance is caused by steric hindrance, bacteriophage may still be able to keep relocating on the host cell surface to find the correct receptors.

O antigen prevents T4 bacteriophage adsorption with specific interference. The presence of O antigen extends the total length of LPS with repeating sugar units of O antigen at the distal end (6). The sugar groups that consist of O antigen may interact with bacteriophage tail fibers during the process of recognition and binding (Fig. 5D). Competitive binding of T4 tails to the distal O antigen molecules of LPS could inhibit bacteriophage interaction with the cell surface receptors that either prevents or slow down the rate of infection. Dimou *et al*. concluded T4 resistance with the constant amount of phage T4 in the supernatant of DFB1655 L9, however, they incubated bacteria and phage only up to 25 minutes (17). If there was enough time given and the interaction between phage T4 and O antigen was reversible, then T4 may be able to reach its host receptors over some period of time.

O antigen expression blocks T4 replication after viral genome injection. Another possible explanation of T4 resistance acquisition in DFB1655 L9 could be the inhibition of viral replication in the cytoplasm (Fig. 5E). The presence of WbbL, a rhamnose transferase, may regulate mechanisms that prevent viral gene expression or assembly in the host cytoplasm. For example, WbbL may interact with the viral replication protein and block the binding with viral DNA. This could potentially be tested by incubating purified O antigen or WbbL with bacteriophage DNA under controlled conditions. However, previous studies showed that the amount of free bacteriophage particles in the supernatant of DFB1655 L9 incubated with T4 was greater than MG1655 (15, 17). Therefore, these findings seem to support a mechanism of inhibition that prevents viral entry into the cell (13).

O antigen expression blocks T4 induced cell lysis. Another potential model is the blocking of T4-mediated cell lysis (Fig. 5F). Here, the injection and replication of viral genome is completed, but the assembled progenies cannot escape from the host due to the incapability of breaking host cell membrane. This can be examined by transforming DFB1655 L9 with T4 genome and observe how the infection progresses. Again, previous observations support the T4 bacteriophage infection is inhibited at extracellular level, so the idea of preventing cell lysis seems less likely (15, 17).

DISCUSSION

E. coli K-12 is a commonly used lab strain, which has lost its ability to synthesize O antigen due to decades of passaging (11). When O antigen synthesis is restored, *E. coli* exhibits increased resistance to bacteriophage infections (11-20). In this review, I summarized the findings from several studies that investigate O antigen mediated resistance to bacteriophage as summarized in Table 1 (11-20). Many mechanisms have been proposed, both extra- and intracellular. However, many findings suggest that O antigen mediated resistance may occur at the cell surface level (14, 15, 17-19).

One research group has attempted to silence *wbbL* expression in DFB1655 L9 to determine if the level of resistance towards T4 would decrease (20). However, transforming DFB1655 L9 with an antisense RNA that targets *wbbL* mRNA did not affect the observed resistance to T4 (20). In this study, Chow *et al*. failed to verify that their new construct reduced *wbbL* translation and, therefore, it is difficult to say if their construct truly was silencing *wbbL* in DFB1655 L9. Another assumption that Chow *et al*. made was that an oversaturation of T4 in the environment would overcome the steric hindrance of O antigen, thus at high concentrations of bacteriophage T4 the resistance may decrease (20). However, DFB1655 L9 remained resistant to T4 even at high MOI, whereas MG1655 was susceptible to T4 at all MOI (20). The idea of silencing *wbbL* translation can be further investigated to understand the exact role of WbbL protein during O antigen synthesis and the resultant phenotype in DFB1655 L9.

Here, the bacteriophage infection resistance by O antigen production in the engineered *E. coli* K-12 strain DFB1655 L9 is described. With the development of isogenic strain of MG1655 with restored O antigen synthesis, the ability of bacteriophage resistance was tested with different absorption assays. Moreover, the possible mechanisms behind such resistance are addressed and suggested. With further elucidation of O antigen-induced bacteriophage resistance of *E. coli*, the potential therapeutics to combat pathogenic *E. coli* could be developed. For example, a phage therapeutic can be raised with the modification of

bacteriophage tail fibers to possess a higher affinity towards a specific *E. coli* O antigen and prevent colonization of pathogenic *E. coli* cells in human body. Since there are various *E. coli* O antigen serotypes that has yet been studied, a further verification of the role of O antigen in *E. coli* will provide more details and new insight into how these findings can be used to pursue benefits in human life.

TABLE 1 Investigation of O antigen-mediated phage resistance in *E. coli* DFB1655 L9 in chronological order. With the restoration of O antigen synthesis in DFB1655 L9, an isogenic strain of MG1655, resistance to different types of phage was observed, and the mechanism behind this resistance was investigated by the series of research groups as described.

Year	Reference	Findings
2013	Browning *et al*.	• Restoration of O16 antigen biosynthesis in *E. coli* K-12 resulted in DFB1655 L9 • DFB1655 L9 is resistant to P1 bacteriophage • DFB1655 L9 is pathogenic to *C. elegans*
2017	Chiu *et al*.	• DFB1655 L9 is resistant to bacteriophage T4 • Resistance is not due to irreversible binding of *E. coli* secreted factors to T4
	Wachtel *et al*.	• DFB1655 L9 is resistant to bacteriophage T4 • Presence of O antigen may prevent T4 adsorption to DFB1655 L9 surface (higher amount of T4 gene detected in MG1655+T4 supernatant than DFB1655 L9+T4 supernatant as measured by qPCR)
2018	Lee *et al*.	• DFB1655 L9 is resistant to both bacteriophage T4 and T7
2019	Dimou *et al*.	• Investigated T4 absorption ability to MG1655 or DFB1655 L9 via double agar overlay plaque assay: confirmed that bacteriophage concentration in MG1655 supernatant decreased overtime, but stayed constant in DFB1655 L9
	Biparva *et al*.	• Visualized interaction between T4 and MG1655 or DFB1655 L9 • Observed T4 integrating with MG1655 whereas no T4 binding on DFB1655 L9
	Morgan *et al*.	• Visualized interaction between T4 and MG1655 or DFB1655 L9: more T4 binding to MG1655 than DFB1655 L9 or JW2203-1 (OmpC deletion) • O antigen production or absence of OmpC is not sufficient to confer long term resistance to T4
	Chow *et al*.	• Silencing *wbbL* translation with antisense RNA did not make DFB1655 L9 susceptible to T4 • DFB1655 L9 is resistant to T4 even at a high MOI

ACKNOWLEDGEMENTS

I would like to thank Dr. David Oliver and Dr. Evelyn Sun for their continuous support and guidance through my manuscript writing. I would also like to thank the Microbiology and Immunology Department in the University of British Columbia for giving me the opportunity to work on course-based undergraduate library research project. Lastly, I would like to thank my colleagues for bearing my situation over the writing period. I would also like to thank two anonymous reviewers for constructive feedback on this manuscript.

REFERENCES

1. **Washizaki A, Yonesaki T, Otsuka Y**. 2016. Characterization of the interactions between *Escherichia coli* receptors, LPS and OmpC, and bacteriophage T4 long tail fibers. *Microbiology Open* **5**:1003-1015.
2. **Yu F, Mizushima S**. 1982. Roles of lipopolysaccharide and outer membrane protein OmpC of *Escherichia coli* K-12 in the receptor function for bacteriophage T4. *J Bacteriol* **151**:718–722.
3. **Chung W, Siu J, Tanner K.** 2005. Roles of lipopolysaccharide and the outer membrane protein OmpC on bacteriophage T4 infectivity in *Escherichia coli*. *UJEMI* **7**:62–67.
4. **Salmond GPC, Fineran PC**. 2015. A century of phage: past, present, and future. *Nat Rev Microbiol* **13**:777-786.
5. **Raetz CR, Whitfield C**. 2002. Lipopolysaccharide endotoxins. *Annu Rev Biochem* **71**:635–700.

6. **Hong Y, Reeves P**. 2014. Diversity of O-antigen repeat unit structures can account for the substantial sequence variation of Wxz translocases. *J Bacteriol* **196**:1713-1722.
7. **Islam MZ, Fokine A, Mahalingam M, Zhang Z, Garcia-Doval C, van Raaij MJ, Rossmann MG, Rao VB**. 2019. Molecular anatomy of the receptor binding module of a bacteriophage long tail fiber. *PLoS Pathog* **15**(12): e1008193.
8. **Wang A, and K Lin**. 2001. Effect of mutations in the outer membrane components on bacteriophage T4 adsorption to *Escherichia coli*. *UJEMI* **1**: 47-53.
9. **Blattner FR, Plunkett G, Bloch CA, Perna NT, Burland V, Riley M, Collado-Vides J, Glasner JD, Rode CK, Mayhew GF, Gregor J**. 1997. The complete genome sequence of *Escherichia coli* K-12. *Science* **277**:1453–1462.
10. **Hobman JL, Penn CW, Pallen MJ**. 2007. Laboratory strains of *Escherichia coli*: model citizens or deceitful delinquents growing old disgracefully? *Mol Microbiol* **64**:881–885.
11. **Browning DF, Wells TJ, França FLS, Morris FC, Sevastsyanovich YR, Bryant JA, Johnson MD, Lund PA, Cunningham AF, Hobman JL, May RC, Webber MA, Henderson IR**. 2013. Laboratory adapted *Escherichia coli* K-12 becomes a pathogen of *Caenorhabditis elegan*s upon restoration of O antigen biosynthesis. *Mol Microbiol* 87:939–950.
12. **Liu D, Reeves PR**. 1994. *Escherichia coli* K12 regains its O antigen. *Microbiology* **140**:49–57.
13. **Ho TD, and Waldor MK**. 2007. Enterohemorrhagic *Escherichia coli* O157:H7 gal mutants are sensitive to bacteriophage P1 and defective in intestinal colonization. *Infect Immun* **75**: 1661–1666.
14. **Chiu J, Croft C, Ng K**. 2017. *Escherichia coli* O antigen serotype O16 is a restriction factor for bacteriophage T4 infection. *UJEMI+* **3**:38-44.
15. **Wachtel A, Guo A, Sagorin Z, Etti E**. 2017. O16 serotype O antigen expression in *Escherichia coli* K-12 may confer resistance against T4 bacteriophage infection by preventing adsorption. *UJEMI+* **3**:70-79.
16. **Lee S, Bie B, Ngo J, Lo J**. 2018. O16 antigen confers resistance to bacteriophage T4 and T7 but does not reduce T4/T7 adsorption in *Escherichia coli* K-12. *UJEMI* **22**:1-11.
17. **Dimou J, Lu J, Pflueger S, Toombs E**. 2019. Rescue of O16 antigen expression in *E. coli* strain MG1655 prevents adsorption of T4 bacteriophage. *UJEMI+* **5**:1-7.
18. **Biparva NM, Ghazizadeh A, Hoang T, Sun S**. 2019. Negative stain electron microscopic analysis suggests O antigen expression in *Escherichia coli* strain K-12 may prevent T4 interactions with the bacterial surface. *UJEMI* **24**:1-11.
19. **Morgan G, Lim D, Wong P, Tamboline B**. 2019. Electron microscopy to visualize T4 bacteriophage interactions with *Escherichia coli* strain DFB1655 L9, an isogenic derivative of strain MG1655 engineered to express O16 antigen. *UJEMI* **24**:1-13.
20. **Chow D, Clark A, Huang YH, Ng SZ**. 2019. Developing the antisense silencing model for the investigation of the mechanism of resistance of *Escherichia coli* DFB1655 L9 to T4 bacteriophage. *UJEMI* **24**:1-11.
21. **Rakhuba DV, Kolomiets EI, Dey ES, Novik GI**. 2010. Bacteriophage receptors, mechanisms of phage adsorption and penetration into host cell. *Polish Journal of Microbiology* **59**:145.
22. **Abedon ST**. 1992. Lysis of lysis-inhibited bacteriophage T4- infected cells. *J Bacteriol* **174**:8073-8080.
23. **Kropinski AM**. 2009. Measurement of the rate of attachment of bacteriophage to cells, p. 151-155. In Clokie MRJ, Kropinski AM (ed.), *Bacteriophages: Methods and Protocols* **Volume 1**: Isolation, Characterization, and Interactions. Humana Press.
24. **Knirel YA, Prokhorov NS, Shashkov AS, Ovchinnikova OG, Zdorovenko EL, Liu B, Kostryukova ES, Larin AK, Golomidova AK, Letarov AV**. 2015. Variations in O-antigen biosynthesis and O-acetylation associated with altered phage sensitivity in *Escherichia coli* 4s. *J Bacteriol* **197**:905–912.
25. **Letarov AV, Kulikov EE**. 2017. Adsorption of bacteriophages on bacterial cells. *Biochemistry (Moscow)* **82**: 1632–1658.