Effectiveness of T4 bacteriophage detachment methods from *Escherichia coli* C149 host cell surface

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*Escherichia* surface molecules, lipopolysaccharide (LPS) and Outer membrane protein C (OmpC), play an important role in the binding of T4 bacteriophage. In order to study the potential role of these two surface molecules on downstream processes of T4 bacteriophage infectivity, experimental methods are required for phage detachment from its host cell surface. In this experiment, we sought to identify an effective method of phage detachment. *E. coli* C149 was incubated with T4 bacteriophage at optimal adsorption conditions and then T4 was detached before a full cycle of replication could occur. The phage bound to the *E. coli* cell surface were subjected to one of three detachment methods including exposure to a pH gradient (pH 3, 5, 8, and 10), extreme temperatures (83°C, 86°C and 90°C) and mechanical shear forces generated while passing the phage bound to their host cells through a QIAshredder column. Phage detachment was monitored by following $^{14}$C-thymine labelled phage and non-radiolabeled *E. coli* with or without treatment with the DNA injection inhibitor DNP. The stability of the *E. coli* cells was monitored by a control which subjects $^{14}$C-thymine labelled *E. coli* to each detachment method. Our results indicate that the method that most effectively detached T4 bacteriophage from *E. coli* C149 host cells while maintaining the integrity of those cells was incubation at 86°C for between 10 to 20 minutes. Alkaline pH also causes phage detachment, but induces cell lysis at the same time. The QIAshredder method proved ineffective as a phage detachment method.

In light of the prevalence of pathogenic bacteria with multiple resistance to antibiotic treatment, alternate therapies are actively sought in the medical community. One such therapy is “phage therapy”, which exploits the relationship between the bacteriophage and its host (5). The T4 bacteriophage, a member of the T-even phage group, is often used as a paradigm in such “phage therapy” research. A full and comprehensive understanding of the T4 bacteriophage lifecycle is therefore necessary, yet incomplete to date.

The T4 bacteriophage consists of a head, a baseplate, and a tail. The head contains double stranded viral DNA, which is injected into the host, *Escherichia coli*, in order to propagate viral infection (4). The tail connects to the baseplate, which attaches to long and short tail fibers responsible for host cell recognition and attachment (6). At the beginning of the infection process, attachment to a host cell receptor involves the interactions between at least three long tail fibers and a glucose residue on the outer core of the lipopolysaccharide (LPS) located on the outer membrane surface of the host cell (1). After this initial attachment phase, the baseplate then interacts with the host cell surface, causing a conformational change which allows the short tail fibers to eject and bind irreversibly to the heptose section of the inner core of the LPS (1). The tail also contains the tail sheath and tail tube which functions to penetrate the outer membrane and inject viral DNA into the host cell cytoplasm (4). The phage particle remains attached to the outer cell surface during the infection, while the injected viral genome shuts down the cells own DNA and RNA synthesizing pathway and replicates its own genome (5). Phage components are also produced by the host’s metabolic machinery. The mature phage components then begin to assemble themselves and give rise to new infectious phage particles. The host cell undergoes lysis and phage progeny is released to infect other cells (6).

Previous studies have demonstrated that the host cell components, LPS and OmpC, are required for T4 bacteriophage infectivity in *E. coli* (15). LPS is a large molecule that contains both lipids and carbohydrates. They are a major superstructure of Gram-negative bacteria which contribute greatly to the structural integrity of the bacteria. LPS is comprised of three parts: polysaccharide (O) side chains; core polysaccharides; and lipid A. Lipid A contains unusual fatty acids and is inserted into the outer membrane while the rest of the LPS projects from the surface (16). OmpC is an outer membrane protein that is associated with regulating the response to the osmolarity of the environment (16). LPS and OmpC have been previously shown to play a role in the binding process of infection, however, their involvement in downstream infection processes, such as DNA injection, remain unclear (1).
In order to investigate the DNA injection phase of the T4 bacteriophage lifecycle, it would be useful to have a method which enables the detachment of phage from *E. coli* after irreversible phage binding has occurred. As there are limited studies on effective methods for phage detachment after irreversible binding, we determined an effective method for T4 bacteriophage detachment from wildtype *E. coli* C149 host cells. We compared three potential methods including a mechanical detachment method (QIAshredder columns), a pH detachment method and a heat detachment method.

Identifying an assay that successfully detaches T4 phage from *E. coli* C149 cells will provide a necessary tool in designing experiments to further analyze the involvement of LPS and OmpC in the bacteriophage lifecycle.

**METHODS AND MATERIALS**

**Bacterial and phage strains.** Wildtype *Escherichia coli* C149 and T4 bacteriophage were provided by the MICB 421 Teaching Lab (Brock Chen) of the University of British Columbia in the Microbiology and Immunology Department.

**Stock solutions.** Ten milliliters of 1 μg / μl non-radioabeled thymine (10 mg thymine added to 10 ml H2O). Five milliliters of 1 M 2,4-dinitrophenol DNA injection inhibitor (0.9255 g 2,4-dinitrophenol DNA injection inhibitor). Two litres Phage broth (30 g peptone, 16 g tryptone, 16 g sodium chloride, 2 g D-glucose, 2000 ml water, pH 7.2). One litre of PBA (13 g tryptone, 8 g sodium chloride, 2 g sodium citrate, 3 g D-glucose, 7.52 g nutrient agar, 1000 ml water, pH 7.2). One litre of PTA (13 g tryptone, 8 g sodium chloride, 2 g sodium citrate, 1.3 g D-glucose, 15 g nutrient agar, 1000 ml water, pH 7.2).

**Preparation of non-radioabeled T4 bacteriophage.** *E. coli* C149 was grown overnight in 20 ml of Phage broth in a shaking water bath at 37°C. The overnight *E. coli* culture was then diluted to 0.15 OD600 with Phage broth and grown in a shaking water bath at 37°C to reach 0.35 OD600. One hundred mililiters of T4 phage stock with an unknown titer was added to the *E. coli* C149 culture and grown in the same conditions until complete lysis occurred and the media appeared clear of culture, which took approximately two hours. Two hundred fifty mililiters of chloroform was added and the sample was centrifuged at 8000 x g for 5 minutes at 4°C. The supernatant was recovered and 100 μl chlorofrom was added. The phage stock was then stored at 4°C.

**Overlay plaque assay.** The phage stock was serially diluted to 10, 10⁻¹, 10⁻², 10⁻³, 10⁻⁴, and 10⁻⁵ by adding appropriate volumes of phage stock in 16 x 150 mm test tubes. One litre of PBA was added to each of the six tubes containing non-radioabeled phage, radiolabeled phage and radiolabeled *E. coli* C149 production. Phage stock was added to the flask intended for the non-radioabeled and radiolabeled phage at an MOI of 1:100. To the flask intended for radiolabeled phage and *E. coli* C149, 8 μg of non-radioabeled thymine (2 μg / ml) and 2.5 μCi / ml of ¹⁴C-thymine (10 μCi / flask) were added. Radiolabeled phage was incubated at 37°C in 200 ml shaking water bath until media cleared reaching 0.1 OD600. Once clearing occurred, 250 μl chloroform was added to the radiolabeled phage and incubated for 5 minutes. The lysate was aliquoted into four 1.5 ml centrifuge tubes and centrifuged at 10,000 x g for 5 minutes. The supernatant was recovered and saved for dialysis. Radiolabeled *E. coli* C149 was centrifuged in a 30 ml centrifuge tube at 10,000 x g for 5 minutes. The supernatant was discarded into a discard flask. The pellet was resuspended with 4 ml Phage broth and centrifuged at 10,000 x g for 5 minutes. The supernatant was discarded and this process was repeated. The pellet was resuspended in 4 ml Phage broth and stored at 4°C.

**Dialysis of ¹⁴C-thymine labeled T4 bacteriophage.** The membrane (Spectra/Por Cat# 132650) was boiled in water for 10 minutes, and then one end was clipped to form a bag. The ¹⁴C-thymine labeled T4 bacteriophage was transferred into the membrane bag. The open end was then sealed with another membrane clip. The membrane bag was submerged in a beaker containing 500 ml of Phage broth and 250 μl of chloroform. Solution was incubated overnight at 4°C with gentle agitation. After 24 hours, the media was mixed with 500 ml of fresh Phage broth with 250 μl chloroform. The dialyzed phage were stored at 4°C (12).

**Monitoring ¹⁴C-thymine incorporation.** Twenty 50 μl samples from the radiolabeled phage and radiolabeled *E. coli* were taken at 20, 40, 60, 80, 90, 110 and 120 time points during the infection process described previously and loaded onto a 3mm paper filter (Whatman 3mm Cat# 1030021). One filter from each time point was air dried at room temperature on the pin board, and the second was immersed in 100 ml of 5% TCA in a 200 ml conical flask over ice. After 10 minutes after the last filter was added to the flask, the 5% TCA solution was decanted into a discard flask. Fifty milliliters of 5% TCA was added to the filters. The filter was swirled and chilled on ice for 5 minutes. The 5% TCA was then decanted and 50 ml of 95% ethanol was then added to the filters for 5 minutes over ice. The ethanol was discarded. Filters were placed on an aluminum tray and baked for 3 hours at 100 – 105°C.

**Mixing Ecoli C149 cells with ¹⁴C-thymine labeled T4 bacteriophage.** For each detachment method described below, ¹⁴C-thymine labeled phage particles were allowed to adsorb to *E. coli* C149 cells for 5 minutes before detachment was performed. Potential detachment was observed by following the location of the phage particles through the measurement of radioactivity in different fractions of the sample-the supernatant and the pellet.

**Controls for detachment conditions.** Controls were set up for each of the detachment methods described below. Radiolabeled *E. coli* C149 cells were exposed to each of the detachment methods in the presence of non-radioabeled T4 phage. Potential escape of *E. coli* C149 cell components were quantified by measuring the amount of radioactivity in the supernatant portion of the samples.

**Heat detachment experiment.** The controls were prepared by transferring 500 μl of radiolabeled *E. coli* C149 into four 1.5 ml eppendorf tubes. To the first tube, 300 μl of Phage broth was added and used as the room temperature control. To the remaining three tubes, 300 μl of 2 x 10⁸ pfu / ml non-radioabeled phage was added to each and used as the 83°C, 86°C and 90°C controls. To another set of six 1.5 ml eppendorf tubes, 1 ml of non-radioabeled *E. coli* C149 at 1.0 OD600 was added to each tube. To three of the tubes, 1.12 μl of Phage broth 2,4-dinitrophenol DNA injection inhibitor was added for temperature points of 83°C, 86°C and 90°C. 120 μl of 1 x 10¹⁰ pfu / ml ¹⁴C labeled phage stock was added to each of the six tubes containing non-radioabeled *E. coli* C149. All samples were incubated at room temperature for 5 minutes to allow for adsorption to occur. The ten tubes were then exposed to their respective temperatures using heating blocks set to either 83°C, 86°C and 90°C.
or 90°C. A 180 µL sample was taken from each tube at 5, 10, and 20 minute time points and a 360 µL sample at the 30 minute time point and placed in a separate 1.5 ml eppendorf tube.

Once all samples were collected, the tubes were centrifuged for 1 minute at 14,000 rpm. One hundred fifty microlitres of the supernatant was loaded onto a 3 mm paper filter disc. The discs were immersed into 100 ml of 5% TCA. An additional 150 µL was removed from the 30 minute time point, loaded onto a 3 mm paper filter disc and left on the pin board to air dry at room temperature. The pellets were washed twice with 800 µL of Phage broth. The remaining cells were resuspended in 100 µL Phage broth and loaded onto a 3 mm paper filter disc.

**pH detachment experiment.** The controls were prepared by transferring 100 µL of radiolabeled *E. coli* C149 into five 1.5 ml eppendorf tubes. To all five tubes, 100 µL of 2 x 10^9 pfu / ml non-radiolabeled phage was added to an MOI of 20:1 and incubated for 5 minutes at room temperature to allow for adsorption to occur. Each tube was adjusted to the desired pH. For pH 3, 9.14 µL of 1 M HCl was added; for pH 5, 2.34 µL of 1 M HCl was added; for pH 8, 3.68 µL of 1 M NaOH was added; and for pH 10, 17.68 µL of 1 M NaOH was added to the appropriate control tubes. The samples were then incubated for 5 minutes at room temperature.

In a separate set of eight 1.5 ml eppendorf tubes, 150 µL of non-radiolabeled phage and 1 ml of non-radiolabeled *E. coli* C149 was added to each of the eight tubes. Thirty microlitres of 1 x 10^7 pfu / ml 14C labeled phage was added to each of the eight tubes for an MOI of 20:1. Each tube was adjusted to the desired pH. For pH 3, 8.23 µL of 1 M HCl was added; for pH 5, 2.11 µL of 1 M HCl was added; for pH 8, 3.31 µL of 1 M NaOH was added; and for pH 10, 9.14 µL of 1 M NaOH was added to the appropriate tubes to end up with two tubes for each pH, one with and one without DNA injection inhibitor. The samples were then incubated for 5 minutes at room temperature.

Once all samples were collected, the tubes were centrifuged for 1 minute at 14,000 rpm. One hundred eighty microlitres of the supernatant was loaded onto a 3 mm paper filter disc. The pellets were washed twice with 800 µL of Phage broth. The remaining cells were resuspended in 100 µL Phage broth and loaded onto a 3 mm paper filter disc.

**QIAshredder detachment experiment.** The control was prepared by transferring 40 µL of 14C-thymine labeled *E. coli* C149 into a 1.5 ml eppendorf tubes. Eighty six microlitres of 1 x 10^9 pfu / ml non-radiolabeled phage was added to each of the two tubes. Thirty microlitres of 1 x 10^7 pfu / ml 14C labeled phage was added to each of the two tubes for an MOI of 20:1. Each tube was adjusted to the desired pH. For pH 3, 2.4-dinitrophenol was added to one of the two tubes. The pellets were washed twice with 800 µL of Phage broth. The remaining cells were resuspended in 100 µL Phage broth and loaded onto a 3 mm paper filter disc.

**RESULTS**

*E. coli* C149 and T4 phage titer and radioactivity. The titer of the non-radioactive phage stock was determined to be 2 x 10^11 pfu / ml. The titer of the 14C-thymine labeled phage stock was determined to be 1 x 10^15 pfu / ml and contained 1.79 x 10^6 DPM / pfu. The titer of the 14C-thymine labeled *E. coli* C149 stock was estimated to be 1 x 10^9 cfu / ml and contained 26, 915 DPM / ml.

**QIAshredder detachment experiment.** The radioactivity in the supernatant of the 14C-thymine labeled *E. coli* C149 was similar for the control as well as the QIAshredder treatment (Table 1). Results for DNP and non-DNP treatments were similar for both the supernatants and pellets.

**Table 1:** QIA Shredder Experiment: Location of radiolabeled bacterial DNA in treatments containing labeled C149 and unlabeled T4 phage, and location of radiolabeled phage DNA in treatments containing unlabeled C149 and labeled T4 phage.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Supernatant (DPM)</th>
<th>Pellet (DPM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Radiolabeled <em>E. coli</em> C149</td>
<td>2203</td>
<td>770</td>
</tr>
<tr>
<td>Control (untreated radiolabeled <em>E. coli</em> C149)</td>
<td>2854</td>
<td>203</td>
</tr>
<tr>
<td>Radiolabeled T4 phage</td>
<td>950</td>
<td>29</td>
</tr>
<tr>
<td>Radiolabeled T4, phage DNP</td>
<td>964</td>
<td>37</td>
</tr>
</tbody>
</table>

-Radioactive counts in pellet were adjusted for a 150 µl sample of resuspended cells. Radioactive counts in supernatant were adjusted for a 150µl sample.

**pH detachment experiment.** As pH increased, radioactivity decreased in the pellet and increases in the supernatant (Fig. 1). As pH increases, radioactivity increases in the supernatant for radiolabeled *E. coli* C149 cells (Fig. 2). Radioactivity ceases to increase in the supernatant as the pH rises above pH 8.

**Heat detachment experiment.** Radioactivity counts in the supernatant of 14C-thymine labeled *E. coli* C149 remain relatively constant over time. A slight rise of radioactivity in the supernatant is observed for the 90°C treatment, and possibly in the 86°C treatment (Fig 3). The radioactivity in the supernatant increases with temperature from 83°C to 90°C. Radioactivity in the supernatant increases over time at 83°C, 86°C and 90°C (Fig. 4). The radioactivity in the supernatant of the DNP treatments is higher than the radioactivity in the supernatant of the non-DNP treatments (Fig. 4).
Fig 1. Amount of DNA leakage from $^{14}$C-thymine labeled *E. Coli* C149 caused by varying pH as measured by amount of radioactivity in cell pellet vs. supernatant.

Fig 2. Desorption of $^{14}$C-thymine labeled T4 phage from *E. coli* C149 at varying pH.
Fig 3. Amount of DNA leakage from $^{14}$C-thymine labeled E. Coli C149 caused by varying temperatures as measured by amount of radioactivity released into supernatant.
Fig 4. Desorption of $^{14}$C-thymine labeled T4 phage bound to *E. coli* C149 at varying temperatures in the presence and absence of DNA Injection Inhibitor. Figure A is at 83°C, B is at 86°C, C is at 90°C.
DISCUSSION

In our experiment $^{14}$C-thymine labeled T4 bacteriophage were allowed to adsorb to E. coli C149 cells. Potential detachment was observed by following the location of the phage particles through the measurement of radioactivity in different fractions of the sample.

For each detachment method two treatments were devised. In one treatment $^{14}$C-thymine labeled phage were allowed to adsorb to cells in the presence of the phage DNA injection inhibitor, 2,4-dinitrophenol (DNP), while in the other treatment the inhibitor was not included. The treatments were then subjected to one of the detachment assays, after which the concentration of radiolabeled phage was monitored in the supernatant as well as in pelleted cells.

For the treatment where DNP was added, it would be expected that since the $^{14}$C-thymine phage DNA remained in the phage head, the radioactivity would be recovered in the supernatant but not the pellet if the assay successfully caused phage detachment. On the other hand if phage remained attached to host cells, it would be expected that the $^{14}$C-thymine contained in the phage that bound irreversibly would be associated with the pellet, while an equal amount would be absent from the supernatant. Assuming that at a low MOI 100% of the phage that attached irreversibly to cells injected their DNA in the treatment where DNP was not included, separation of the phage from host cells will not be detectable since DNA is no longer associated with the viral particles. Furthermore, it would be expected that the same result would be obtained as in the case where the DNA injection inhibitor was added but separation was not achieved. This is because the same number of phage should bind to cells irreversibly in either treatment. (See Table 2 for an illustration of the theoretical experimental outcomes).

Table 2. Theoretically possible outcomes of phage detachment experiments: Theoretical distribution of radioactivity derived from radiolabeled T4 phage DNA in the supernatant and pellet.

<table>
<thead>
<tr>
<th></th>
<th>DNP absent</th>
<th>DNP present</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phage Detachment</td>
<td>No Phage Detachment</td>
</tr>
<tr>
<td>Supernatant</td>
<td>X - Y</td>
<td>X - Y</td>
</tr>
<tr>
<td>Pellet</td>
<td>Y</td>
<td>Y</td>
</tr>
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</table>

“X” is the initial radioactivity contained in the supernatant before phage binding occurs.

“Y” is the radioactivity contained in the phage that bound irreversibly to cells.

The results indicate that the QIAshredder detachment experiment does not result in the lysis of E. coli C149 cells, as the radioactivity in the supernatant of radiolabeled cells that were subjected to QIAshredder treatment does not exceed the radioactivity in the supernatant of untreated cells (Table 1).

The fact that there are around four fold more radioactive counts in the pellet of the treated radiolabeled cells than the untreated radiolabeled cells can be attributed to a procedure generated artifact, which can be explained as follows. The control contains less cells than expected since the analyzed aliquot of sample was taken from a cell suspension that was not properly resuspended, while the pellet derived from the QIAshredder treated cells represents the centrifuge precipitation of all the cells that were present in the sample. Thus, the amount of cell material in the control was underrepresented. It was shown that the control was not resuspended before aliquots to be analyzed were taken, as the obtained pellet size steadily decreased over four time points (data not shown).

It was hypothesized that when E. coli C149 cells pass through the small pores in the QIAshredder column at high speed, shear forces would cause $^{14}$C-thymine labeled phage (MOI of 4.3:1) to detach from the cells. However, when the treatment with DNP and the treatment without DNP are compared, it can be seen that the radioactivity in both the supernatant and the pellet are very similar between the treatments (Table 1). This suggests that the shear forces generated in the QIAshredder column were not sufficient to cause phage detachment from host cells, since phage that bound to host cells appear to have remained associated with the pellet in the treatment that contains the DNA injection inhibitor.

$^{14}$C-thymine labeled E. coli C149 was used to determine the stability of E. coli C149 cells in the presence of T4 bacteriophage (MOI 20:1) under each pH used in the treatment (Fig. 1). At pH 3, all detected radioactivity was found in the pellet. At pH 5, the amount of radioactivity in the pellet was 72% of the total amount detected. At pH 6.8, the amount of radioactivity in the pellet declines to 30% of the total amount of radioactivity detected. The radioactivity in the pellet then nears 0% as the pH approaches pH 10.

The decline in cell stability at the more alkaline pH’s is expected due to the instability of cell walls of Gram-negative bacteria at basic pH’s above pH of 8. This instability results in the release of plasmid and sheared chromosomal DNA (8). The fact that cell components are being released into the supernatant is also demonstrated by the increase in measured radioactivity in the supernatant observed as pH increases from pH 6.8 to 10.
A previous study has shown that at pH 2.0, *E. coli* growth is not inhibited until after six hours of incubation at 37°C and cell lysis is not expected to occur prior to this time (11). The actual counts obtained at pH 3 in the pellet were unexpectedly low, most likely due to an error in our procedure (Fig. 1). The pellet was exposed to various wash and resuspension steps that may have resulted in a partial loss of the pellet. The lack of radioactivity in the supernatant in the supernatant is an indication that while a portion of the cells had not lysed at pH 3. The decrease in radioactivity in the supernatant of the treatments with DNP and the treatments without DNP is that radioactivity in the supernatant increases as pH increases to pH 8, after which further release of radioactivity into the supernatant ceases.

In the case of cells treated with DNP, the trend can be explained as follows. As the pH increases, phage that bound to cells and did not inject DNA due to DNP, are desorbing from cells until most phage particles have detached at pH 8. This observation is supported by the literature which indicates that desorption of T4 bacteriophage increases as pH also increases (11). Further increasing the pH to 10 does not result in a significant change, suggesting that most phage have desorbed by pH 8.

In the case of cells that were not treated with DNP, the trend can be explained by taking into consideration that the MOI is high. With an MOI of 20:1, non-radiolabeled *E. coli* C149 host cells are expected to be bound by multiple 14C-thymine labeled T4 phage. Due to the superinfection exclusion phenomenon, the phage that inject their DNA first inhibit DNA injection of the other phage that are bound to the same host cell (5). The result is that the majority of bound phage retain their 14C-thymine labeled DNA in the treatments without DNP. The phage that had retained their radioactivity will be released into the supernatant along with the phage that had successfully injected their DNA into the host cell. Therefore, as pH increases to pH 8, the treatments without DNP follow a similar trend to treatments with DNP.

Between pH 6.8 to pH 8, the data suggests that the treatments with DNP have a consistently higher radioactivity count in the supernatant than the treatments without DNP. This confirms that some phage in the treatments without DNP have injected their DNA into host cells, and that the DNA that was injected into *E. coli* stays associated with the cells. As the pH increases above 8, the values for the radioactivity in the supernatant of the treatments with DNP and the treatments without DNP begin to converge, which suggests that cells are lysing and that the radiolabeled DNA injected into cells in the treatments without DNA is beginning to escape into the supernatant. It is likely that the data points at pH 10 have been erroneously reversed between the DNP and non-DNP treatments.

Decreasing pH below 6.8 appears to favor adsorption, as seen by the decrease in radioactivity in the supernatant (Fig. 2). Acidic pH values can therefore not be used to cause phage desorption from *E. coli* cells.

The radioactivity recovered in the supernatant of radiolabeled *E. coli* C149 incubated at room temperature remained relatively constant over time at around 2900 DPM, if the 30 min time point is attributed to experimental error (Fig. 3). This value represents the amount of radioactivity already present in the supernatant of the radiolabeled cells before they were subjected to treatment, and can be considered background. Radiolabeled cells at 83°C have a similar radioactive count over time, which suggests that cells are not caused to leak at this temperature. At 20 min, the 83°C radioactive count is much lower than expected, but this can probably be attributed to a pipeting error.

The radioactivity in the supernatant for both the 86°C and 90°C treatments are slightly greater than that of the room temperature treatment, suggesting that minimal leaking occurs at these temperatures. Leaking appears to occur at a constant rate over time and the rate of leaking seems to increase with temperature.

Figure 4 describes the effects of temperature over time on phage detachment at an MOI of 12:1 by displaying the radioactivity in the supernatant originating from 14C-thymine labeled T4 phage. Due to the superinfection exclusion phenomenon, the phage that inject their DNA first inhibit DNA injection of the other phage that are bound to the same host cell (5). The result is that the majority of bound phage retain their 14C-thymine labeled DNA in the treatments without DNP. The phage that had retained their radioactivity will be released into the supernatant along with the phage that had successfully injected their DNA into the host cell. Therefore, as pH increases to pH 8, the treatments without DNP follow a similar trend to treatments with DNP.

At each of the measured temperatures for both the treatment with DNA injection inhibitor and the treatment without DNA injection inhibitor, the data implies a very rapid initial rate of phage detachment between 0 and 5 min (Fig 4). This initial rate of detachment appears to increase with increasing temperature. At 90°C, the data implies that this initial
rapid rate of phage detachment ceases before the 5 minute time point is reached (Fig 4C).

As in the pH experiment, the high MOI in this experiment resulted in the treatments without DNP resembling the phage detachment trends shown by the treatments with DNP, due to the superinfection exclusion phenomenon. The 20 minute data points at 90ºC were likely erroneously switched between the DNP and non-DNP treatments (Fig. 4C).

At 83ºC and at 86ºC, maximum phage detachment is achieved by 10 minutes, and does not change significantly after 30 minutes of incubation (Fig. 4A and Fig. 4B). At 90ºC, maximum phage detachment is achieved by 30 minutes according to the data, but this could be as a result of experimental error (Fig. 4C).

The difference between the amount of radioactivity in the supernatant of the DNP and non-DNP treatments at 83ºC, 86ºC and 90ºC between 10 and 30 minutes after start the start of incubation is around 250 DPM, 500 DPM and 120 DPM respectively (Fig. 4). This suggests that 86ºC is the optimal desorption temperature. Possibly 86ºC causes greater detachment of phage from cells than 83ºC, which would increase the radioactivity in the supernatant of the DNP treatment relative to the non-DNP treatment. 90ºC may cause cells to leak slightly, which would release radiolabeled DNA injected into non-DNP treated cells, increasing the radioactivity in the supernatant of the non-DNP treatment relative to DNP treatment.

The method that most effectively detached T4 bacteriophage from E. coli C149 host cells while maintaining the integrity of the cells was heat treatment with 86ºC for between 10 to 20 minutes. While treatment with basic pH also caused desorption, cell lysis was observed to occur, making this method less desirable for the purpose of investigating T4 phage DNA injection.

FUTURE EXPERIMENTS

The results of our investigation show that heat can be used to detach T4 phage from E. coli C149 cells without causing significant cell lysis, and that 86ºC is a more optimal temperature than 83ºC or 90ºC. However, further studies should be conducted in order to optimize the method.

A similar experiment to the one conducted in this study should be carried out that will test temperatures at 1ºC intervals between 83ºC and 90ºC. The experiment should be carried out in triplicate in order to make a statistical analysis possible. It would also be beneficial if the 3H-thymine labeled phage were more radioactive, which could perhaps be achieved if less carrier was used than in the protocol employed in this study. This would allow for an MOI of 1:1 to be used, which would prevent the superinfection exclusion phenomenon from obscuring the data obtained. Further methods of detaching phage from E. coli C149 such as changing osmolarity or MgSO4 concentration could also be investigated (11,16).

Once an effective detachment method is determined, this method can be used to further elucidate mechanisms involved in DNA translocation of the T4 bacteriophage into its host cell, E. coli. For example, the importance of LPS and OmpC surface proteins in phage binding can be tested by using radiolabeled phage DNA to infect a culture of E. coli C149 wild type cells as well as E. coli LPS mutants and OmpC mutants.

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