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Development of a Method for Characterization of Bacterial Host DNA Degradation During Nucleolytic Phage Infection – Investigation of Preferential Degradation of Host genomic DNA Using T7 Bacteriophage Infection of *Escherichia coli* K-12

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The T7 bacteriophage lytic life cycle has been thoroughly characterized, however, the possibility of site-specific cleavage of host genomic DNA by T7 nucleases has not been well-investigated and could provide a novel method of probing chromatin structures in bacterial genomic DNA. Previous studies have shown that T7 endonuclease gp3 cleaves branched cruciform DNA structures, the migration of which are known to be blocked by nucleoid proteins composing nucleosomes in the *Escherichia coli* genome. Here we describe a method for investigating the stability of DNA regions known to bind heat unstable (HU) nucleoid and integration host factor (IHF) proteins. Host DNA was sampled over the course of *E. coli* K-12 MG1655 infection by T7 bacteriophage and visualized by pulsed-field gel electrophoresis. Potential protected regions were then probed by quantitative polymerase chain reaction. The resulting data indicates a possible persistence of these nucleoid-associated regions during phage infection, which may be confirmed by further optimization of PFGE and qPCR protocols. This experimental approach represents a possible novel means of probing bacterial chromatin structure.

Phage infection is often characterized by nucleolytic degradation of host DNA. Nucleolytic degradation of host *Escherichia coli* DNA by T7 bacteriophage nucleases gp3 and gp6 allow the invasive phage to hijack essential host transcriptional and metabolic resources and causes the release of free deoxyribonucleic acid monophosphates from degraded host DNA to favor phage DNA proliferation (1).
Lee and Sadowski demonstrated that T7 exhibits site-specific cleavage of its own genome in gp6-knockout T7 strains (2), which implies underlying site-specificity in gp3 endonuclease activity. This implication was later substantiated in work conducted by Massy *et al.* that demonstrated *in vitro* site-specific cleavage of branched cruciform DNA structures by gp3 (3). Correspondingly, Grigoriev and Hsieh have used reconstituted histones to show that Holliday junction migration is blocked by nucleosomes in *E. coli* (4). This suggests that there may be areas of host genomic DNA that may be protected from degradation by T7 nucleases.

E. coli's genomic DNA is known to interact with at least twelve endogenous host histone-like structures such as the nucleoid proteins integration host factor (IHF) and histone like protein (HU) that have been demonstrated to influence gene expression and migration of structures facilitating genetic recombination and alteration of DNA structure (4-6).

Although *E. coli* and T7 bacteriophage have both been studied extensively, it remains unknown whether regions of the host genome are protected from degradation during infection, a phenomenon that if substantiated, would provide a means of probing chromatin structure with bacteriophage. In this study, a method for probing the preferential degradation of host *E. coli* K-12 MG1655 genome by T7 bacteriophage was developed. MG1655 was chosen specifically for this project due to its susceptibility to T7 bacteriophage infection. Furthermore, Prieto *et al.* designed primers against regions of DNA associated with HU and IHF proteins in MG1655 during a chromatin immunoprecipitation study. We proposed that visualizing T7 degradation of host genomic DNA over time via pulse-field gel electrophoresis (PFGE) and probing resulting host genomic DNA fragments for specific nucleoid-protein associated regions by qPCR would substantiate preferential degradation or protection of genomic DNA from T7 nucleases.

MATERIALS AND METHODS

***E. coli* K-12 MG1655 and T7 Bacteriophage Strain Preparation**
Bacterial strain *E. coli* K-12 MG1655 and T7 bacteriophage were obtained from the MICB447 culture stock (Department of Microbiology and Immunology, University of British Columbia). The 1Zeta K-12 stock plate was prepared by first growing a culture overnight in Luria Broth (LB) at 37°C, and subsequently streaking for isolated colonies. T7 bacteriophage was amplified by infecting 4.8 x 10^9 cells/ml of *E. coli* K-12 MG1655 and incubating the culture until the culture cleared (OD_{600} of 0.010). Phage particles were then harvested by treating the culture with 15 drops of 0.1% (v/v) chloroform and centrifugation at 10,000 x g for 10 minutes. The supernatant was passed through a 0.45 µm filter to remove any remaining cell debris.

T7 Bacteriophage Time Course Infection. An *E. coli* K-12 MG1655 culture grown to an OD_{600} of ~0.6 in a volume of 15 ml was infected with T7 bacteriophage at an MOI of 5 (Fig.S1) and incubated at 25℃ in a shaking water bath incubator. Immediately after infecting with T7, a 1 ml sample was removed (T_0) and placed in a 2 ml eppendorf tube. 1 ml samples were subsequently removed every 5 minutes up to 55 minutes (T5-T55). In addition, an uninfected 1 ml sample was removed prior to infection.
Immediately following each sample removal, 100 µl of 10% SDS was added and the mixture vortexed briefly. Sample tubes were then moved to a fume hood and 900 µl of 25:24:1 Phenol:Chloroform:Isoamyl Alcohol was added, and mixed by

inversion. Samples were then left to rest until completion of the entire time course sampling.

After collection of the last time course sample (T55), all samples were spun down in a table-top microcentrifuge (Beckman), and DNA was isolated by two rounds of phenol:chloroform extraction. DNA was precipitated by adding 1/10 volume of sodium acetate (pH 5.2, to 0.3 M final) to the final aqueous phase, and 1 ml cold 100% ethanol (50% final ethanol concentration), with mixing between additions. Samples were then incubated at 20°C for >20 minutes, spun down at max speed for 10 minutes, and supernatant decanted. The DNA pellet was washed with 70% ethanol and spun down again, the supernatant decanted, and the pellet allowed to briefly dry (~10 minutes) before resuspension in dH_2O.

DNA concentrations were measured on a Nanodrop 2000 Spectrophotometer and resuspended deionized water to 1000 ng/µl before freezing for future applications at -20°C.

Random Shearing by Sonication . The sonification protocol was adapted from Sanbrook *et al*. (7). *E. coli* MG1655 genomic DNA was extracted using ThermoFisher Scientific Genomic DNA Mini Kit. DNA was eluted using dH_2O instead of elution buffer. The concentration of DNA samples was then measured by Nanodrop 2000 Spectrophotometry to confirm the purity and concentration of DNA samples, using sterile dH_2O as a blank. Sonication was performed using a Braun-Sonic 2000 Ultrasonic cell disruptor at 50 watts in 5 second bursts for up to 10 bursts. Between each interval, the samples were put on ice for 20 seconds and an aliquot of DNA taken after the 20 second chilling period. Each aliquot of DNA was measured again using Nanodrop to confirm DNA concentration. Initial attempts at random shearing by DNase incubation were also attempted, the results of which can be found in Supplementary Materials, Fig. S-2.

Pulsed-Field Gel Electrophoresis (PFGE). T7 Time Course DNA samples were visualized by PFGE. Optimization was performed to ensure good separation of fragments (Fig. S-3). DNA samples were suspended 1:1 in 2% Low-Melting Point Agarose gel plugs at 55°C (40 µl total volume). DNA plugs were embedded in the wells of a 1% agarose gel (0.5X TBE) and the gel was run using the BioRad CHEF-DR® II system, including the electrophoresis cell, drive module, control module, variable-speed pump and cooling module. Switch time was set at ramping from 60-120 seconds (linear ramp), Voltage at 5.5 V/cm, and a run time of 20 hours, maintained at 14°C. An *S. cerevisiae* chromosomal DNA standard (0.225 - 2.2 Mb) (BioRad) was used as a molecular weight ladder. Bands were visualized by Ethidium Bromide staining.

Quantification of degradation of host genome regions bound by histone-like proteins by qPCR. Quantitative polymerase chain reaction (qPCR) was used to determine the extent of degradation of protein-bound genomic regions. As DNA is degraded, it is expected that the amount of intact template that can be amplified will decrease. Three types of regions, which have been previously shown to interact with histone-like proteins, were targeted: regions bound by histone-like (HU) protein, integration factor alpha (IHF-α), and integration factor beta (IHF-β) (4-6). Primers targeting these regions were adopted from a chromatin immunoprecipitation sequencing (ChIP-Seq) experiment in which enrichment of regions bound by these three proteins was further validated by qPCR (6). Twelve primer sets were chosen based on annealing temperature, amplicon length, and region targeted. The sequences of all twelve primer sets are available in supplemental table 1.

Confirmation of amplicon formation by polymerase chain reaction. In order to confirm that primers would not amplify phage genome, primer sequences were BLAST-ed against all T7 genomes using NCBI-BLAST blastn (http://blast.ncbi.nlm.nih.gov/Blast.cgi). To confirm that amplicons could be successfully produced from host *E. coli* MG1655 genomic DNA, PCR was conducted with the twelve primer sets, and visualized on a 2% SYBR-Safe (ThermoFischer Scientific) DNA agarose gel (Fig. S4) PCR mixtures consisted of 10 µl of 2X SsoAdvanced ™ SYBR® Green Supermix (BioRad, cat. no. 1725265, Hercules CA), 4 µl of 5 µM forward and reverse primer mix, 2 µl of 52.24 ng/µl purified *E. coli* MG1655 genomic DNA, and 4 µl sterile dH_2O. Cycling was conducted using the BioRad CFX Connect Real Time System and was programmed as follows: denaturation at 95°C for 3 minutes, 35 cycles at 95°C for 15 seconds and 55°C for 20 seconds, and held at 4°C indefinitely. Two primer sets targeting regions bound by each of HU (primer sets, IHF-α, and IHF-β and two reference primer sets targeting non-nucleoid protein bound regions were chosen for qPCR. Sequences are as given in supplemental table 1.

Protocol for qPCR targeting nucleoid-bound regions of T7-infected host genomic DNA. qPCRs were conducted in triplicates with DNA extracted at each of the time points in the infection time course experiment. Two negative controls were included: ddH_2O only, and T7 DNA only. Primers were previously tested without DNA template and were shown to not form dimers (Fig. S4). Two positive controls were also included: uninfected *E. coli* MG1655 DNA, and sonication-sheared DNA averaging 500 bp in size**.** *E. coli* MG1655 DNA (described in Methods, above). A standard curve was conducted with primer set HU-1 to determine the optimal template concentration to be 2 ng/reaction (Fig. S5). PCR mixtures consisted of 10 µL of 2X SsoAdvanced ™ SYBR® Green Supermix (BioRad, Cat. no. 1725265), 4 µl of 5 µM forward and reverse primer mix, 2ng DNA template and was topped up to 20 µl with sterile dH_2O. qPCR was conducted using the CFX™ Connect Real-Time Detection System (BioRad), and cycling conditions were as previously described.

Statistical analyses and normalization of qPCR data. qPCR data analysis first involved removal of outliers within triplicates. Outliers were identified based on substantial differences from other replicates in the set Ct averages (difference of > 1 Ct), and standard deviation was re-calculated after outlier removal. qPCR results required normalization for differences in primer set efficiency and PCR DNA input in order to compare trends in template degradation during the infection course across all primer sets. Therefore, the qPCR data was first normalized against the intact *E. coli* MG1655 positive control by calculating the ΔCt of each infection timepoint sample for each primer set. The ΔCt is defined as $Ct_{(time\ point)}$-$Ct_{(positive\ control)}$. Subsequently, the data was normalized against the ΔCts of two reference primer sets as each time point had a slightly different DNA input due to dilution inconsistency. This should normalize for within time-sample differences in DNA input. Samples were normalized against its respective time point reference Ct values. ΔΔCts were calculated for each primer set against both references at all infection time points, and was defined as follows: $\Delta\Delta Ct = \Delta Ct_{(target)} - \Delta Ct_{(reference)}$. Fold difference were calculated as follows: fold difference = $2^{-\Delta\Delta Ct}$. Figures were generated using Excel. Certain data points were removed due to outliers affecting normalization or high replicate standard deviations, as documented in supplemental table 2.

RESULTS

Sonication breaks down host genomic DNA into fragments of approximately 200-800bp. Sonication was used as a means to generate randomly sheared host genomic DNA fragments for use as a positive control in qPCR experiments against T7-infected time course samples (Fig. 1). Following sonication, each aliquot of DNA was run on a 1% agarose gel Fragments resulting from the degradation of MG1655 genomic DNA by T7 were too large for

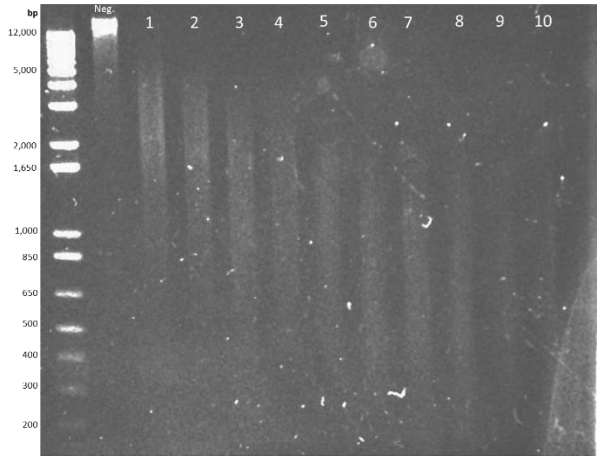

FIG 1. Sonication of MG1655 genomic DNA. Neg. refers to negative control - an un-sheared genomic DNA sample. Lane number represents the number of 5 second sonication bursts DNA underwent.

visualization by standard gel electrophoresis, PFGE was utilized instead.

PFGE of T7 time course DNA shows fragments consistent in size with the T7 genome. In order to visualize DNA fragments resulting from degradation by T7 bacteriophage endonuclease, 5 minute time-point DNA samples were collected over the course of a 1-hour T7 infection of *E. coli* K-12 MG1655 at 25℃ and visualized by PFGE (Fig. 2). The *E. coli* culture was infected with T7 at an MOI of 5, previously determined to be sufficient for ensuring complete infection of bacterial cells (Fig. S1). DNA was isolated from each time point by phenol-chloroform extraction and ethanol precipitation. Total lysis of the *E. coli* culture occurred just past the 1 hour mark, indicating successful infection by T7 at the sub-optimal temperature of 25℃ (contrasted with the typical 17 minute life cycle at 37℃). DNA samples were then run on a PFGE gel, at parameters set to target resolving of 0.6 - 1.5 Mb fragments. Samples were run alongside whole T7 genomic DNA and whole MG1655 genomic DNA, in addition to an *s. cerevisiae* chromosomal DNA standard. As expected, the T7 genomic DNA yields a single band of <225,000 bp (T7 genome is 40,000 bp), while the MG1655 genomic DNA appears to have been unable to migrate down the gel (MG1655 genome is ~4 Mb). Each time course sample showed bands similar in size to the T7 genomic DNA control, with the exception of T35, which showed no band at all. Samples from time points T15 on show noticeable smearing around the band, indicating some further fragmentation of the DNA. DNA appearing in wells may be due to air bubbles between the DNA-agarose plug and the well walls during loading. PFGE resolved the large DNA fragments resulting from the time course infection, and yielded bands that appear to be composed primarily of T7 genomic DNA, likely stemming from infecting T7 along with newly-synthesized T7 genomic DNA during infection. However, the true source of the fragmented DNA is impossible to ascertain using only visualization on a gel. Therefore, qPCR probing various nucleoid-specific regions of the host genome was pursued.

Average threshold cycle values (Ct) show similar trends over time for each primer set. A similar trend could be observed across all nucleoid-associated region-specific primer sets throughout the infection course (Fig.3). This occurred regardless of the region of the host genome amplified during PCR. The average Ct peaks at 10 minutes after infection, decreases to a minimum by 20 minutes after infection, and then increases steadily until the end of the infection course. Whereas one might expect the amount of intact template to decrease over time due to T7 nuclease activity, this trend is not observed. Although averaged Ct values increase from 20 minutes onwards, this trend towards higher Ct averages is not consistent across time points. Ct values would increase and decrease irregularly. This along with the observation that Ct averages peak at around 10 minutes suggests the existence of possible confounding factors, such as DNA input per reaction. Additionally, different primer set efficiencies are suggested by the values presented in Figure 3. For example, averaged Ct values are consistently higher for IHFa-1 than that for HU-Ref. This suggests that certain primer sets more efficiently annealed to its respective template than others at the PCR conditions given in this experiment. Alternatively, differences in Cts could have arisen from inherent properties of the different primer sets. For example, a primer set yielding a larger amplicon may have a lower Ct value as a fluorescence signals can reach its threshold sooner with more DNA to intercalate

Template stability trends are differential between nucleoid-region-specific primer sets. As shown in Figure 4, each primer set exhibited a different trend in template stability over the infection course following normalization. When normalized against HU-Ref, a slight decrease in

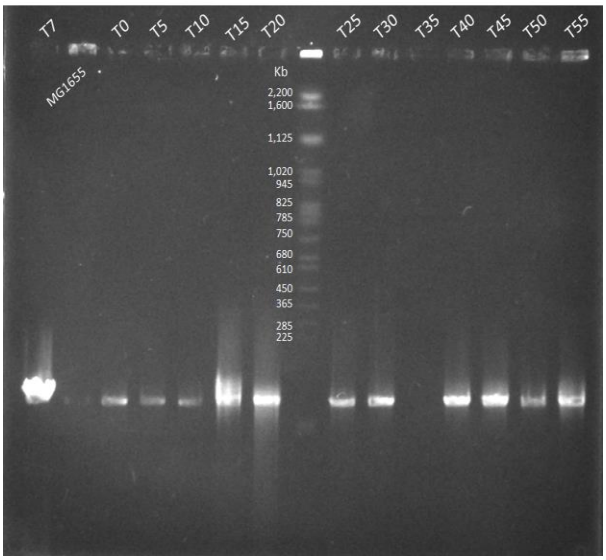

FIGURE 2. T7 time course DNA samples resolved by PFGE. Whole T7 genomic DNA and whole MG1655 genomic DNA were run as controls (lanes 1 and 2). 1% Agarose gel was run in 0.5X TBE for 20 hours, switch time ramped from 60 - 120 sec, voltage at 5.5 V/cm.

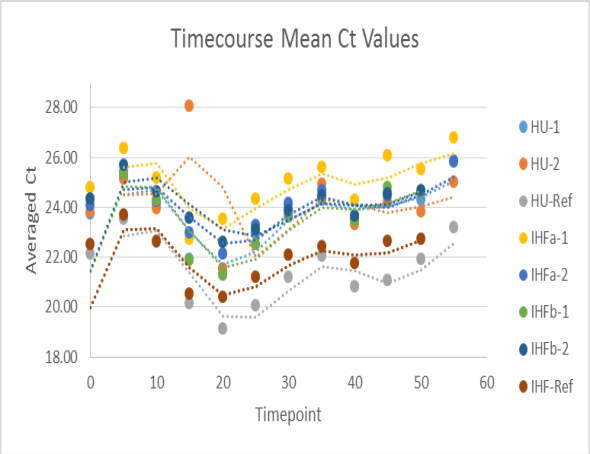

FIG 3. Trends in average Ct values from qPCR across all primer sets over the infection time course. Data is presented as un-normalized, and only data points that were outliers (difference of >1 Ct) were removed from average calculations.

template bound by HU proteins can be observed (Fig. 4a). This trend is also observed for IHF-bound regions, though the trend line observed in available template is more irregular (Fig. 4b). When normalized against IHF-Ref, there appears to be no discernable pattern. The templates associated with HU-1, HU-2, IHFa-1 and IHFb-2 seem similarly abundant to the reference (Fig. 4c-d). However, IHF-a2 and IHF-b1 template availability peaks between 15-20 minutes, and IHFb-1 exhibits a much higher fold-difference in template than all other primer sets (Fig. 4d). These results are contrary to expectations that there would be no difference in template availability at the beginning of infection as the host genome has yet to be degraded, and suggests the existence of additional confounding factors and that the reference primer sets chosen were unsuitable accounting for PCR DNA input differences. Furthermore, template stability trends differed for a given primer set when normalized against a different reference primer set.

Reference primer sets show different template preservation trends over T7 infection time course. Inconsistencies between template preservation trends when normalizing against different reference primer sets suggests that the references sets used may not have been ideal (Fig. 4). these reference primer sets may have been unsuitable as their respective template may also be degraded over the infection course. Consequently, reference Ct values may not serve as a good indicator of DNA concentration, and may be unsuitable for normalizing the different DNA concentrations of time course samples. Similar differences in template stability trends were also observed when either of the reference primer sets were normalized against each other (Fig. 5). If both reference sets behaved similarly, similar trends should be observed when either sets are normalized against each other. Instead, similar trends are only observed up until 15 minutes post-infection, and then diverge. This suggests that the two reference sets behave differently, or that unaccounted confounding factors may be affecting the qPCR.

DISCUSSION

The purpose of this study was to develop a method of characterizing *E. coli* host DNA during nucleolytic infection by the T7 phage. The characterization of host DNA degradation was attempted from the angles of visualization of DNA degradation banding patterns by DNA pulsed-field gel electrophoresis and quantifying known nucleoid protein-associated genomic regions. However, the results of this experiment do not definitively assess whether certain regions in the host genome are preferentially preserved or degraded during infection as various inconsistencies in experimental results cannot yet be explained.

Visualization of DNA extracted at various infection points by pulsed-field gel electrophoresis was inadequate for definitively characterizing host DNA degradation. Trace amounts of large DNA fragments were observed in wells across all time points (Fig. 2), and it is uncertain whether this represents intact host genomic DNA or large fragments of degraded host genomic DNA that cannot be resolved by experimental gel conditions. Alternatively, these large residues could be the result of air bubbles within the agarose plug blocking the migration of certain DNA fragments. Consistent with our expectations, a large band corresponding to the size of the T7 genome becomes more intense approximately 15 minutes post-infection (Fig. 2). However, this band is poorly resolved across many lanes, and appears as large smears in certain lanes. Time course sample concentrations were normalized before loading equal volumes in each well. However, it is possible that contaminating RNA masked true DNA concentrations when measured by spectrophotometry, and therefore unequal DNA amounts were loaded in wells. Alternatively, since a minimum concentration of 3 ng/band is required for visualization on a gel when using SYBR® Safe DNA gel stain, it is possible that DNA fragments of different sizes may be present, but in too low concentrations to be visible on the gel. The poor resolution and the irregular changes in intensity of this band suggest that some wells were overloaded with DNA, and hence limit the ability to characterize DNA degradation. As the smear associated with the T15 band stretches upwards to 365 kb, whether or not host genomic fragments larger than the size of the T7 genome were produced cannot be discerned. Furthermore, visualization by standard gel electrophoresis does not allow T7 DNA to be discerned from potential fragments of host DNA. Altogether, a better approach towards visualizing DNA degradation needs to be developed.

Our approach in assessing host genomic DNA fragmentation does not take into account the possibility that degradation may be focused at particular sites, such as near the viral replication site. If the host DNA degradation is centralized at only particular regions of

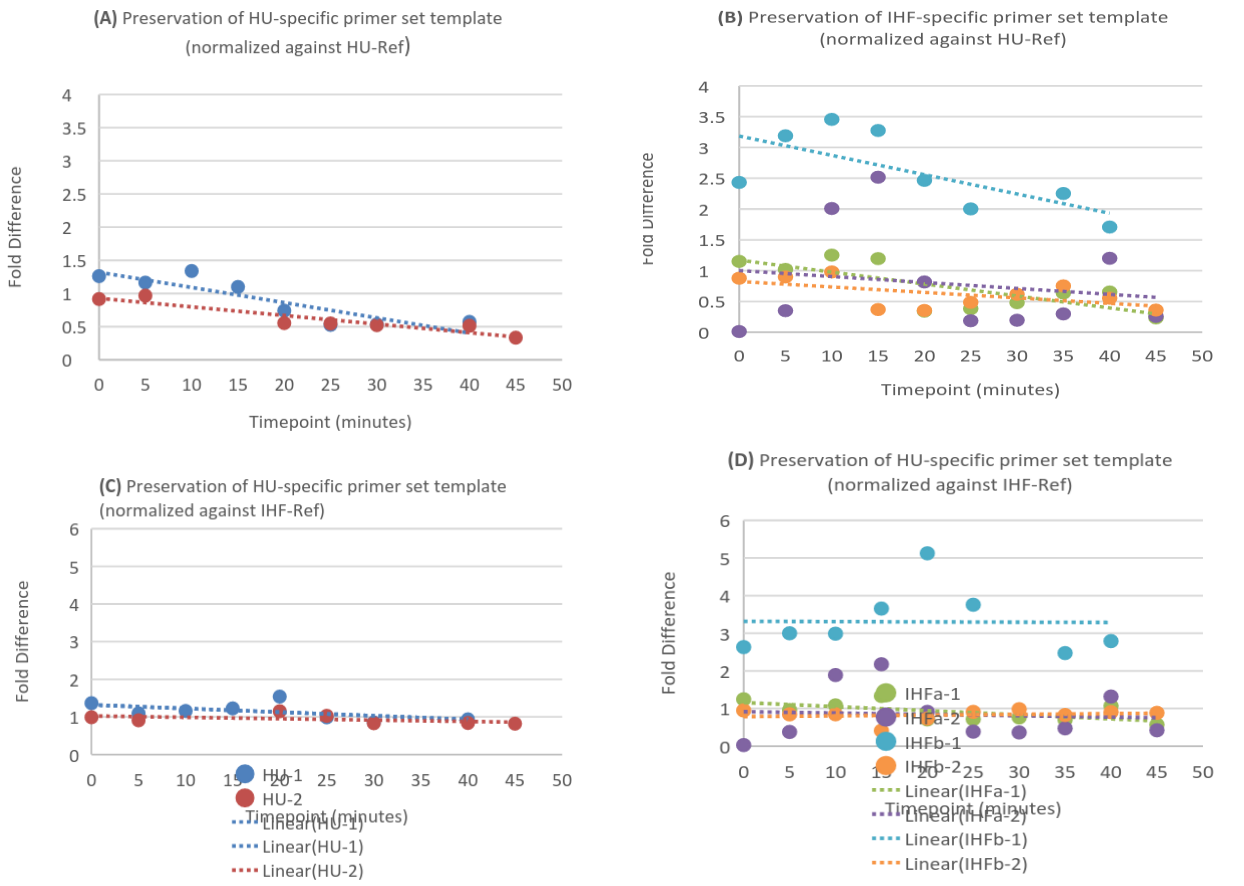

FIG 4. Trends in template preservation targeted by nucleoid-protein bound primer sets over the course of infection. Represented by fold difference to (a-b) a non-HU bound region and (c-d) a non-IHF bound region. Fold difference was determined from ΔΔCts from qPCR data and high variability data points were removed as described in Methods. Trend lines represent moving average difference in qPCR data across time points.

the cell, it may be likely degraded fragments would be too short to be detected and leave only an almost full length bacterial genome. Our approach using PFGE would not be sensitive enough to pick up minor difference in genome fragment size. An alternative approach, such as whole genome sequencing.

The qPCR results hint at different trends in template degradation, but whether or not this is an experimental artifact remains to be determined. Figure 3 shows fold differences normalized to reference primer sets in Figure 4 that indicate highly variable patterns in template preservation. Certain primer sets exhibit much higher fold differences across time points than other primer sets (Fig. 4d), while other primer sets exhibit very similar trends in template stability. For example, IHB-1 exhibited a much higher fold difference than all other primer sets. Furthermore, there are noticeable differences when a given primer set is normalized against a different reference. While normalization against a reference DNA set is standard when analyzing qPCR data, the references used in this study were inappropriate as normalizing reference sets, as previously discussed. In conjunction with a cross-comparison of the two reference primer sets (Fig. 5), these results suggest that the selected reference primers may not have been suitable for accounting for different DNA inputs at each time point. As the reference primers target regions of the host genome, it may be possible that targeted templates may become degraded over the course of infection or sample preparation, making it a poor indicator for DNA input. Rather, it may be more suitable to compare trends in template preservation of reference primer to that targeted by nucleoid-protein bound primer sets. However, this cannot be effectively compared without controls for primer efficiency and DNA concentration variability.

The qPCR results are further confounded as negative controls with T7 genomic DNA indicated the presence of amplifiable template (Supplementary table 2). This result suggests contamination of the phage stock with bacterial DNA, as these primer sets did not yield any T7-specific BLAST hits. While treating the phage stock with nuclease should have degraded any DNA from previous bacterial hosts, the qPCR results suggest that nuclease treatment may have been ineffective. It is possible that

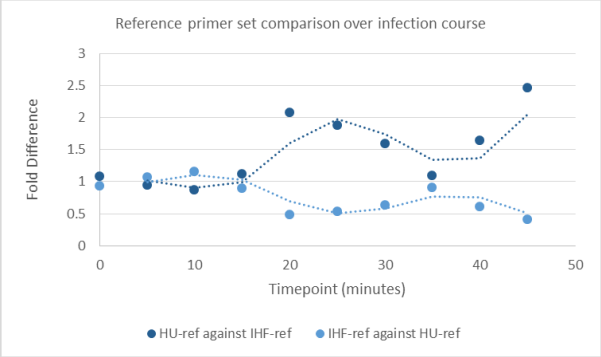

FIG 5. Cross-comparison of template preservation of reference primer sets. Represented by fold difference. Fold difference was determined from ΔΔCts from qPCR data and high variability data points were removed as described in Methods.

the qPCR primers are amplifying DNA from the T7's previous host, rather than bacteria infected during the time course, which would further confound the results of qPCR. Alternatively, host DNA may have been packaged within the phage capsid through the process of transduction

Furthermore, the results of qPCR seem inconsistent with observations on DNA degradation by pulsed-field gel electrophoresis. Gel electrophoresis indicates accumulation of DNA fragments that are approximately the size of the T7 genome at 15 minutes post-infection and comparatively very little large host-genome sized fragments retained in each well (Fig. 2). However, qPCR results indicate consistent levels of template across the infection duration (Fig. 3). On one hand, this result could suggest that the smears observed at approximately the size of the T7 genome include fragments of host DNA. However, this cannot be definitely established as residues of very large fragments can be observed in the wells of each lane (Fig. 2). As each qPCR had a targeted DNA input, the ratio of T7 phage DNA to large fragments retained in the gel well should be reflected by qPCR Ct values. However, this is not the case. For example, the T7 band is the brightest at 15 minutes (Fig. 2) but its respective Ct values for each of the primer sets is among the lowest (Fig. 3). Whether or not result implies the presence of host genomic fragments within the T7 band is confounded by the lack of normalization for qPCR input DNA.

Although an MOI of 5 was found to be sufficient for total cell lysis at 37°C, since the time course infection experiment was carried out at 25°C, it is possible that the decreased temperature impacted the adsorption of phage particles to host cell surfaces, resulting in a decreased number of total cells infected (8). Uninfected cells could have the following effects on our data: uninfected cells, when lysed during the DNA isolation protocol at each time point, would yield intact genomic DNA. When used as template for qPCR, this could then result in the false appearance of certain DNA regions being protected from T7 degradation. Higher MOIs should be retested at 25°C in order to identify the optimal MOI for achieving total cell lysis at that temperature.

In conclusion, although the data suggests that there is preferential preservation of certain nucleoid-associated regions, this conclusion cannot be substantiated due to the amount of uncertainty when analyzing the data. Pulsed-field gel electrophoresis holds promise as a method of visualizing the degradation of host cell genome during T7 bacteriophage infection, as demonstrated by resolution of bands <225 kb, however further optimization must be performed in order to make it a feasible strategy for identifying discrete fragments that may persist throughout infection. In addition, qPCR has great potential as a means of quantifiably observing the persistence of particular nucleoid-associated regions; it was successful in probing these regions of intact and fragmented host genomic DNA. However again, additional measures of control must be applied in order to conclusively state whether particular regions are enduring throughout infection, as confounding factors such as seemingly amplifiable template within our T7 genomic DNA only control call into question the reliability of our results. Also comment on improvements on the experimental setup.

FUTURE DIRECTIONS

In order to make qPCR an effective method for quantifying the potential persistence of nucleoid-associated regions, a control for amount of DNA template input should be applied. To control for amount of DNA isolated from each time course sample by phenol-chloroform extraction, a known amount of alternate DNA, such as plasmid, could be spiked into each sample prior to DNA isolation. An additional primer specific to this plasmid could then be used in qPCR, a standard curve developed, and the results applied against standard curves for each experimental primer used, to normalize for amount of DNA isolated. Not sure if I like this idea. It introduces several new confounding variables.

PFGE should be repeated with varying conditions in order to optimize the separation of bands in the <225 kb range. Additionally, extra care should be taken with loading DNA-agarose plugs to minimize air bubbles and potential trapping of DNA in wells. By treating DNA isolated samples with RNase, and then repeating spectrophotometry DNA concentration measurements, a more accurate loading of DNA samples into the gel could be accomplished, to achieve equal DNA loaded throughout all samples.

In order to determine the source of DNA within smeared bands in PFGE, a Southern Blot could be performed with probes designed against protein-bound regions of the host genome and T7 genome. As well, to minimize the possibility of contaminating host genomic DNA within T7 genomic DNA controls, nuclease activity during T7 isolation should be tested, to ensure effective degradation of residual host genomic DNA is occurring.

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