Journal of Experimental Microbiology and Immunology (JEMI) Vol. 11:66-72

Effects Of Microwave Irradiation And Heat On T4 Bacteriophage Inactivation

SYLVIE BRYANT, RONAK RAHMANIAN, HERMAN TAM, AND SABA ZABETIAN
Department of Microbiology & Immunology, UBC

Two theories have been previously described that potentially explain how T4 bacteriophage are inactivated using microwave sterilization: thermal and non-thermal effects. In order to investigate the mechanism of inactivation following microwave irradiation, purified T4 bacteriophage were subjected to microwave irradiation for 10, 15, and 20 seconds (thermal effect) and for 10, 20, 30 and 40 seconds while on ice (non-thermal effect). In addition, T4 bacteriophage samples were heated to 65°C, 80°C and 95°C for 10 minutes to determine the effect of heat alone. The treated samples were subsequently analyzed via standard bacteriophage plaque assays to determine survival. DNA agarose gel electrophoresis was performed to identify samples with ruptured versus intact capsid and to identify any possible phage DNA mutagenesis due to the treatments. Non-thermal microwave irradiation of T4 phage resulted in minor killing while thermal microwave irradiation showed significant killing, likely due to capsid rupture. External heat showed notable killing as well, though it was likely that the DNA itself was denatured. Although the data presented in this study support the thermal theory of inactivation, the exact mechanism of bacteriophage inactivation following microwave irradiation remains inconclusive.

Microwave sterilization of various materials have been studied for decades and reported inactivation of microorganisms include *Candida albicans* (20), *Lactobacillus casei* (7), *Bacillus subtilis* (12), *Escherichia coli* (15), and bacteriophages (MS2, T4, T7, PL-1, lambda phage) (7, 11, 12). There are two proposed mechanisms of microwave inactivation: thermal effect and non-thermal effect. The theory behind the thermal effect is that energy is transferred from microwaves to the specimen with a consequent rise in temperature (3). Heating allows phage particles to absorb the electromagnetic energy and transforms it into thermal energy through atomic level vibration until a threshold temperature, where bonds between amino acid break and denature proteins of the phage particles (3). In contrast, the non-thermal effect attributes inactivation in the absence of temperature increase due to electroporation, formation of membrane pores, and magnetic field coupling (12, 5, 17). Electroporation forms membrane pores due to the electron potential across the membrane causing leakage of cellular materials (5). Rupturing of the capsid, as a result of voltage drop, causes DNA release and tail breakage resulting in inability for host cell adsorption (12). Electromagnetic energy coupling with essential cellular components such as protein or DNA disrupts internal activities and leads to cell lysis (2). Both theories have the potential to generate atomic motion causing phage capsid and phage tails to become unstable and subsequently break (12).

There is evidence supporting both the thermal irradiation and non-thermal irradiation mechanisms of phage inactivation. Some experiments have shown that microwave irradiation resulted in a higher degree of microorganism inactivation compared to conventional heating (4, 10) where as others have shown no difference at all (5, 17). In addition, non-thermal levels of microwave irradiation cause DNA damage by causing single and double stranded breaks in the genome (7, 13).

Previous studies have used T4 and T7 bacteriophage in order to investigate the mechanism of bacteriophage inactivation following microwave or heat treatment (2, 20). However, these studies have conflicting results. Hence, this study was performed to determine whether the thermal irradiation or non-thermal irradiation mechanism inactivates T4 bacteriophage. Non-thermal and thermal irradiation using the microwave, and heat alone were tested. A plaque assay was used to determine bacteriophage survival after treatment with non-thermal and thermal microwave irradiation, and heat alone. In addition, DNA agarose gel electrophoresis was used to determine capsid breakage.

MATERIALS AND METHODS

Bacteria and Phage. Wild type *E. coli* B23 was used as a host for T4 bacteriophage and both were kindly provided from the MICB 421 culture collection in the Department of Microbiology and Immunology at the University of British Columbia.

Host Cell Culture for use in Plaque Assay. A Luria broth agar plate ((tryptone (1.0 g), sodium chloride (1.0 g), yeast extract (0.5 g),

pH to 7.2, agar (1.5 g), distilled water (to 100 ml)) was streaked with *E. coli* B23 and incubated in the dark at 37°C until isolated colonies appeared. *E. coli* B23 culture was prepared via inoculation of 15 ml of Luria broth (tryptone (1.0 g), sodium chloride (1.0 g), yeast extract (0.5 g), pH to 7.2, distilled water (to 100 ml)) with a single isolated *E. coli* B23 colony from the LB plate, and incubated overnight at 37°C.

Phage Titring Plaque (Overlay Technique) Assay. Bottom agar plates ((tryptone (13.0 g), sodium chloride (8.0 g), sodium citrate (2.0 g), glucose (1.3 g), pH to 7.2, agar (15 g), distilled water (to 1L)) were warmed in the 37°C incubator for at least 30 minutes. Autoclaved top agar ((tryptone (13.0 g), sodium chloride (8.0 g), sodium citrate (2.0 g), glucose (3.0 g), pH to 7.2, agar (7.5 g), distilled water (to 1L)) (3 ml in each 13 x 100mm tube) was boiled at 100°C until liquid and kept at 55°C in a water bath until used. T4 bacteriophage was serially diluted with tryptone sodium glucose (TSG) broth ((tryptone (0.05 g), sodium chloride (0.3 g), yeast extract (0.7 g), D-glucose (0.13 g), pH to 7.2, distilled water (to 100 ml)) to 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7}. One hundred microliters of diluted phage sample was added to each top agar tube, which were still kept warm in the 55°C water bath. Two hundred microliters of overnight *E. coli* B23 culture was added to each phage containing-top agar tube, and the tubes were immediately mixed by phage-style mixing and directly poured onto a plate of bottom agar, which was tilted back and forth to ensure that top agar coverage was even. Plates were allowed to solidify for approximately 40 minutes, then inverted and incubated at 37° C for 18-20 hours until plaques formed**.**

Phage Purification. Enough phage was plated via above plaque assay technique so that confluent lysis was just obtained. After adding 1m TSG to each plate, top agar was gently scraped into sterile, screw cap centrifuge tubes using a sterilized flat, metal spatula. Another 1 ml of TSG was used to rinse the remaining top agar and deposited into the same centrifuge tube. One hundred microliters of chloroform (regardless of the amount of phage sample obtained) was added and incubated for 30 minutes at room temperature with periodic mixing. The centrifuge tubes were incubated at 4°C for 72 hours to allow the phage particles to separate from top agar, and then centrifuged at room temperature at ~11,000 g for 10 minutes, or until pellets formed. Supernatants were collected into a similar, sterile tube with 200 µl chloroform and stored at 4°C. The amplified, purified phage suspension was titred by performing serial dilutions to 10^{-8} and using the above described plaque assay plating method.

Non-thermal and thermal microwave irradiation. For non-thermal irradiation, an autoclaved glass petri dish was put into a 1 L beaker of packed ice. Half a milliliter of undiluted, purified T4 bacteriophage suspension was added to the centre of the petri dish and microwaved (Samsung Model MW5592W) at high power for 10, 20, 30, and 40 seconds. Irradiated phage was recovered using a sterile glass Pasteur pipette. The procedure was repeated for the thermal irradiation treatment, but with the removal of the beaker of ice, for 10, 15 and 20 seconds, using 2 ml of T4 bacteriophage instead of 0.5 ml due to evaporation of samples.

Heat treatment. Water baths were set up to 65ºC, 80ºC, and 95ºC. Three sterile 10ml test tubes were each filled with 0.5 ml of undiluted, purified T4 bacteriophage suspension. One test tube was placed in each water bath and the sample was heated for 10 minutes. Phage was recovered using a sterile Pasteur pipette.

Phage survival plaque assay. Each sample from each treatment (thermal and non-thermal irradiation, heat), was titered using the plaque assay method as described above.

Phage DNA Extraction to generate controls for DNA agarose gel electrophoresis. The phage capsid was purposefully disrupted to isolate phage DNA (14). Proteinase K was added to 100 µl of purified T4 sample to a final concentration of 50 µg/ml. Sodium dodecyl sulfate (SDS) (5% w/v in water) was added to a final concentration of 0.5%. The tubes were inverted several times to mix and incubated for 1 hour at 65^0C. An equal volume of equilibrated phenol, (saturated solution (pH 8) Amresco, Lot # 0116 B01) was added and the tubes were mixed by inversion, then centrifuged at 1600 g for 5 minutes at room temperature. The top aqueous phase was transferred to a clean microcentrifuge tube. This aqueous phase was extracted: once with a 50:50 mixture of equilibrated phenol and chloroform (minimum 99%, SIGMA, Batch #055K0070), and once with an equal volume of chloroform, and collected in a clean microcentrifuge tube. Ninety five percent salted EtOH (0.1 volume 2M NaCl) was added in 2.5X the volume of the sample (250 µl), and the tube was mixed by inversion and stored at -80^0C for 20 minutes. Samples were spun at 13000 g for 15 minutes at 4ºC fridge. The supernatant was discarded and the pellet resuspended in 75% EtOH and centrifuged again at high speed for 15 minutes. The supernatant was discarded and the pellets were dried in the eppendorf vacufuge at 30ºC for 30 minutes and then resuspended in 40 µl sterile dH_2O.

Restriction digest and DNA agarose gel electrophoresis. Eighteen microliters of phage sample (control or treated) was added with 2 µl REact4™ digestion buffer (16304-016, Invitrogen) and 2 µl NdeI (15232-036, Invitrogen) restriction enzyme. Samples were placed in a 37ºC heating block for 90 minutes. Sample buffer (6X, bromophenol blue (0.025 g), xylene blue (0.025 g), 50% glycerol (6 ml), 50x TBE buffer (1.2 ml), distilled water (2.8 ml)) was added to samples to 1X to stop the reaction and samples were stored at 4ºC until electrophoresis. Samples were loaded into a 0.4% agarose gel (15510-019, Invitrogen) in 1X TBE (45mM Tris borate (pH 8.5) 1mM EDTA). Lambda phage DNA digested with Hind III (15612-013, Invitrogen) was used as the DNA ladder. Electrophoresis was run in 1X TBE at 100 V for 90 minutes (or until dye front reached bottom of gel). The gel was stained with ethidium bromide at concentration of 0.2 µg/ml for 15-30 minutes and photographed under UV light (AlphaImager, AlphaInnotech).

RESULTS

Survival Plaque Counts on time course of the inactivation of T4 bacteriophage. In order to investigate the effect of non-thermal irradiation, thermal irradiation, and heat alone on T4 phage survival, the percent phage survival following each treatment was determined using standard plaque assay (Table I). The titre of the untreated, purified T4 phage sample was 3.0×10^8 pfu/ml. The percent phage survival of irradiated T4 sample decreased slowly as the irradiation time was increased, with a 24% drop in the 40s irradiated as compared to the 10s irradiated sample. In contrast, the percent phage survival dropped significantly by 91% following 10s of heat and irradiation treatment. The heat treated phage sample also had a similar trend in terms of the drop in the phage survival.

Effects of microwave irradiation on T4 bacteriophage analyzed by agarose gel electrophoresis. In order to determine the effect of non-thermal irradiation, thermal irradiation, and heat alone treatments on the intactness of the phage capsids, T4 phage samples were analyzed by 0.4% agarose gel electrophoresis. An initial gel electrophoresis was performed on microwave irradiated samples alone (Fig. 1). Distinct bands of similar intensity and size of approximately 23kbp were observed in all lanes. However, it was expected that multiple bands be present in each lane due to the activity of NdeI restriction enzyme. In addition, no differences in banding pattern were observed between the treated and untreated T4 phage samples. Therefore, a second

TABLE 1: Percent survival of T4 bacteriophage following non-thermal microwave irradiation, thermal microwave irradiation, and heat treatments. Percent survival was calculated by average titre of T4 bacteriophage after treatment divided by the original titre of T4 bacteriophage (determined via survival plaque assay results), multiplied by 100%.

Treatment	% Survival
Non-thermal irradiation	
10s	99.3
20s	91.2
30s	90.0
40s	75.4
Thermal irradiation	
10s	9.5
15s	0.2
20s	0.0
Heat	
65°C	96.7
80°C	0.0
95°C	0.0

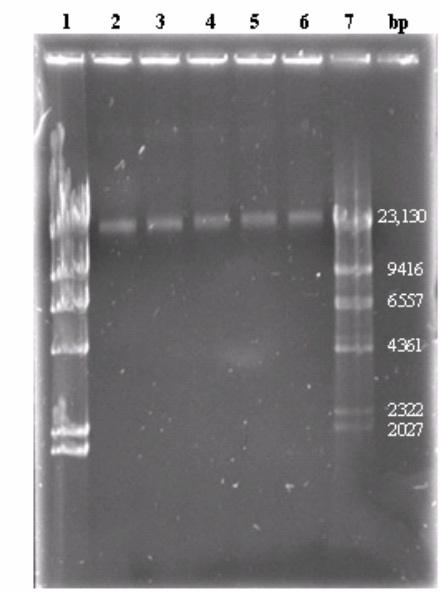

FIG. 1 Electrophoresis of microwave irradiated of T4 bacteriophage in 0.4 % agarose ran at 100 volts for 90 minutes and stained with ethidium bromide (0.2 ug/ml). The samples are untreated T4 phage (lane 2), 10s non-thermally irradiated T4 (lane 3), 20s non-thermally irradiated T4 (lane 4), 30s non-thermally irradiated T4 (lane 5) and 40s non-thermally irradiated T4 phage (lane 6). All samples were digested with NdeI restriction enzyme. HindIII λ DNA marker (15612-013, Invitrogen) was used as a standard (lanes 1 and 7).

agarose gel was run to investigate the effect of each treatment on T4 phage. As shown in figure 2, a common band of similar intensity and size of approximately 23kbp was again present in lanes 2-15. Also, an unexpected smearing was observed in the lower molecular region of several lanes (lanes 2, 4-7 and 18) as well as in the higher molecular region of above 23kbp (lanes 2-4, 6-18).

Several additional controls were used for proper comparison of our samples, including untreated phage ± NdeI restriction enzyme or proteinase K, or ethidium bromide. NdeI restriction enzyme was used to detect the difference in the banding pattern between the intact and digested T4 phage genome but no notable difference was observed. In lanes 2 and 3, at the 23kbp region, two bands of slightly different molecular weight were observed with the lower weight band in lane 2.

Proteinase K was used to intentionally break the phage capsid and release the phage DNA to determine the banding pattern difference and molecular weight of whole, released DNA from an untreated phage sample. However, there was no major difference in the Proteinase K treated samples (lanes 4, 5 and 7) as compared to the Proteinase K untreated samples (lanes 2, 3 and 6) except for a dark streaky band in lane 4 at ~23kbp.

Ethidium bromide changes the conformation of DNA molecules by intercalating between DNA bases and was used to determine the differences in the rate of migration and conformation of the T4 genome. Yet, there was no significant difference observed in the banding pattern of ethidium bromide treated (lanes 6 and 7) and untreated samples (lanes 2 and 4). It must be noted that the approximately 23kbp bands in lane 6 and 7 are slightly of higher molecular weight than the band in lane 2 and 4 respectively. There is no difference observed in the banding pattern of the non-thermally irradiated, thermally irradiated, and heated samples (lanes 8-15). Hence, no conclusion can be drawn in terms of the difference in the effect of each treatment on the intactness of the phage capsid.

DISCUSSION

Microwave irradiation of microorganisms can occur via either thermal or non-thermal factors. The thermal theory proposes that microwaves cause atomic level vibrations which converts to thermal energy that is capable of denaturing capsid proteins, enzymes, and nucleic acids at high temperatures (3). Supporting evidence has been seen in experiments with *E.coli* and *B. subtilis* (5). One study has demonstrated almost complete loss of microwave inactivation of various bacteria, fungi, and bacteriophage (K12) in a dried versus a suspension form suggesting that cell

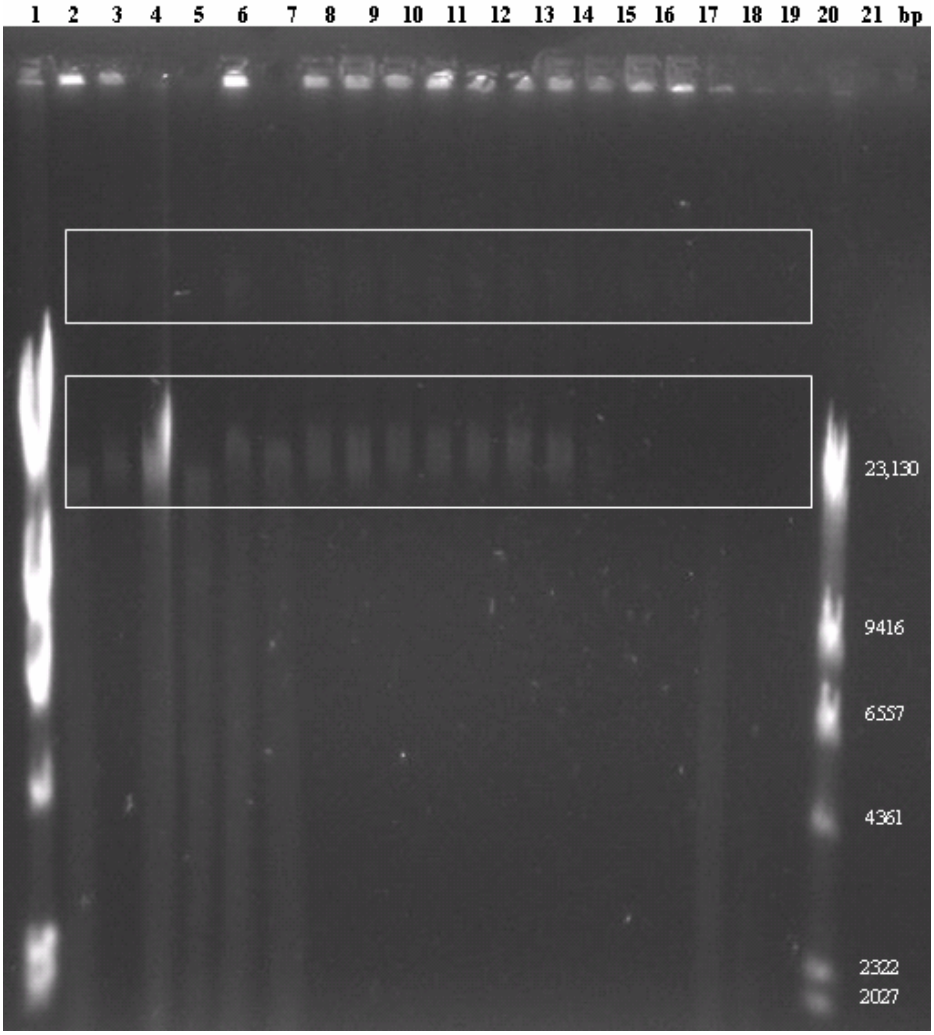

FIG. 2 Electrophoresis of non-thermally irradiated, thermally irradiated, heated, and untreated samples of T4 bacteriophage in 0.4 % agarose ran at 100 volts for 2 hr and stained with ethidium bromide (0.2 ug/ml). The samples are untreated T4 phage (lane 2), untreated T4 digested with NdeI (lane 3), untreated T4 + Proteinase K (lane 4), untreated T4 + Proteinase K digested with NdeI (lane 5), untreated T4 + ethidium bromide (lane 6), untreated T4 + Proteinase K + ethidium bromide (lane 7). Samples in lane 8- 17 are all digested with NdeI: 10s non-thermally irradiated T4 (lane 8), 20s non-thermally irradiated T4 (lane 9, 11), 30s non-thermally irradiated T4 (lane 10, 12), 40s non-thermally irradiated T4 (lane 13), 10s thermally irradiated T4 (lane 14), 15s thermally irradiated T4 (lane 15), 65˚C heated T4 (lane 16), 80˚C heated T4 (lane 17), 95˚C heated T4 (lane 20). The remaining samples are *E. coli* (lane 18), and *E. coli* digested with NdeI (lane 19) and HindIII λ DNA marker (lane 21).

constituents other than water do not absorb enough thermal energy to inactivate microorganisms (17).

As suggested by our source experiment (2), a time course of treatment was performed to analyze trends in microbial inactivation. In contrast to the centrifuge tube-in-tube method used (2, 20), the phage suspension was placed on a glass sterile petri dish without lid to allow for consistent microwave irradiation exposure with a uniform depth that was absent in previous experiments. When the heat factor was isolated via an external ice bath, T4 bacteriophage had over 75% survival rates after 40 seconds of non-thermal irradiation as compared to almost 0% after 20 seconds of thermal irradiation treatment (Table I). The data strongly supports the heat theory in inactivating T4 bacteriophage. Although different volumes were used in the thermal versus non-thermal methods, the effect of volume of phage suspension on survival rates was negligible (20). Another study has shown that thermal microwave was the main determinant in inactivating PL-1 phage as opposed to external heat exposure (7). In this experiment, the three external heat treatments were chosen based on the ability of T4 to survive at temperatures as high as 70^{o}C (20). The greater decrease in survival rate during the heat treatment (Table I) may be due to prolonged external heat exposure (minutes) as compared to the short microwave treatments (seconds). It should be noted that even at 65^{o}C, a

temperature at which T4 bacteriophage is expected to survive for a prolonged period of time (20), a slight reduction in survival rate was observed.

The genome of bacteriophage T4 has some complex and unique features, which should be carefully considered for proper experimentation. T4 phage DNA (a T-even phage) contains the unusual base 5-hydroxymethyl cytosine (5'-OHMC) in place of cytosine, and is further modified through the covalent linkage of glucose residues to a portion of the 5'-OHMC bases; thus enabling the viral abrogation of host restriction defenses (19).

It is this distinctive modification of the T4 phage genome that enforces the careful selection of a proper restriction enzyme for this experiment, as the genome glucosylation prevents cleavage by most enzymes. NdeI restriction nuclease was selected based on the initial research, as it was believed to have the ability to cut unmodified, glucosylated phage DNA (9). Digestion with endonuclease NdeI was expected to produce 67 fragments of genomic DNA in samples whose DNA had been released due to the rupturing of the capsids (9). Microwave or heat treatment were expected to release phage genomic DNA through capsid disruption, making it available for endonuclease cleavage; whereas the DNA of the intact phage of the untreated samples and those with unbroken capsids would have remained protected from restriction enzyme activity (22).

A comparison between lane 2 of figure 2, containing untreated T4 phage, and lanes 8-15, containing non-thermal microwave irradiated, and thermal microwave irradiated phage sample, illustrates a similar banding pattern of a lower 23kb band with a very faint higher molecular band, as highlighted in the results. Two different approaches may be applied in the explanation of these parallel banding patterns. First, it is possible to suggest that the non-thermal microwave irradiation treatment alone was unable to rupture the phage, thus leaving the DNA shielded from digestion within the capsid. It is also likely that even though the phage capsid was broken, the NdeI restriction enzyme was unable to cleave the released genome, due to DNA glucosylation despite our original expectation, resulting in similar banding patterns. The plaque assay results obtained, however clearly support the former analysis, as there was an average rate of 90% survival in the non-thermal microwave irradiation treated samples (Table I).

There are no bands observed in lanes 16, 17 & 20, which contain the heat treated samples (Fig. 2). This may be due to the fragmentation of the phage genome during the destruction of the T4 capsid. In addition, prolonged high temperatures applied in heat treatment may have resulted in the melting of the DNA double strands, which can no longer intercalate ethidium bromide between the bases, resulting in the absence of bands or smears within these lanes.

In order to assess the functionality of the NdeI restriction endonuclease, a proteinase K/SDS extracted phage genome, treated with NdeI was included as a control (lane 5). There appears to be no difference between the banding patterns in this lane and that of the untreated intact phage in lane 2. This suggests that NdeI restriction enzyme was incapable of cleaving the methyl/glucosylated genome of T4 phage. However, further optimization of the NdeI digest conditions may be required to confirm this analysis. Extensive research following these results have led us to believe that EcoRV and TaqI restriction enzymes would have perhaps been more appropriate for cleaving the unique T4 DNA (6, 8).

In figure 2, a great deal of smearing and streaking is observed in all lanes including those of the ladders in lanes 1 and 21. This may have been due to the inappropriate preparation of the gel creating an uneven agarose concentration within the gel. It may have also been the result of the utilization of an unclean gel comb, which may have created a barrier with in the wells, preventing the samples from freely traveling into the gel. In addition to gel quality, the method of storage may have influenced the increase in the observed smearing over time, as noted through the comparison of figures 1 & 2. Although intact T4 phage is believed to be very stable, previous studies have demonstrated a very slow rate of phage denaturation when stored at $4^{o}C$, also claiming that chloroform should not be used for preservation, as it inactivates one third of most tailed and filamentous phages (1). This may explain the presence of smears below the 23kb band in figure 2 (lanes 2-7), which were absent previously (Fig. 1). Furthermore, as the thermally and non-thermally irradiated samples were also stored at $4^{o}C$, they were also subject to denaturation, resulting in a decrease in the distinction of the bands observed on the gel over time.

The T4 viral genome is a linear, dsDNA molecule of about 170 kbp, which is terminally redundant. This terminal redundancy enables the genome to become circular by recombination (19). This may be an important factor to consider when analyzing band patterns in agarose gel electrophoresis. An evaluation of the banding pattern in lane 3 of figure 2, containing the isolated phage genome, illustrates a bright band of about 23 kb and a faint band of higher molecular weight, a pattern similar to that present in most of the other lanes. A possible explanation for this discrepancy between the band sizes observed on the gel and the actual T4 genome size may be the recombination of the redundant terminals of the T4 genome once released from the capsid, forming a circular molecule that is capable of supercoiling. Supercoiled DNA forms two

structures: a plectoneme and/or a toroid, with plectonemes being the form most commonly adopted by free DNA and typically more common in nature (16). Therefore, it is possible to assume that the lower 23kb band is the plectonemic form of the circular T4 genome, while the faint high molecular weight band is the toroid configuration. To test this hypothesis, the untreated phage sample and the SDS/proteinase K extracted phage genome were both treated with ethidium, an intercalating drug, and were ran as controls on the gel (lane 6 & 7, Fig. 2) (16). This treatment was expected to cause inter-conversion among superhelical forms, altering the movement of the DNA during gel electrophoresis (16). Although the 23kb bands in lane 6 and 7 appear to be slightly higher than the band observed in the lane containing the untreated phage samples (lane 2), the difference is fairly minute and the poor quality of the gel along with the smears in figure 2 interfere with proper analysis.

Microwave irradiation is a common method of sterilization. From the survival plaque assay, thermal effect was more significant in inactivating T4 bacteriophage than non-thermal effects. Although this data supports the thermal effect theory, the actual mechanism of capsid breakage remains unclear. Understanding the actual mechanisms can optimize the design of microwave treatment including: duration, volume, equipment material, and temperature. The inactivated T4 bacteriophage may also serve as a model to study the inactivation of food borne pathogens via microwave and to develop safety precautions to food poisoning by susceptible pathogens, such as utilizing microwave sterilization for home equipment like kitchen sponges and plastic scrubbers (12).

FUTURE EXPERIMENTS

Future studies into the mechanism of bacteriophage inactivation should focus on the use of a proper restriction enzyme to analyze the bacteriophage genomic DNA. Optimizing the conditions and experimental protocols of the microwave and heat experiment would allow for a thorough investigation of the combined effect of microwave irradiation and heat on bacteriophage inactivation. One problem that may have affected the survival assay results was the container used in the microwave. The relatively small volume of T4 sample did not spread evenly on the large glass petri dish and may have resulted in varying degrees of microwave exposure so an increased volume can resolve this issue.

In addition, an alternate DNA ladder, with a greater range of molecular weights should be used. The preparation of a larger gel or the reduction of the electrophoresis voltage would also allow an improved resolution of the higher molecular weight bands, and permit enough time for the larger molecular weight fragments, which may have been trapped in the wells, to migrate down into the gel.

ACKNOWLEDGMENTS

This study was supported by the Department of Microbiology and Immunology, University of British Columbia. The authors would like to sincerely thank Dr. William Ramey, Jennifer Sibley, Karen Smith and Po-Yan Cheng for their technical assistance in the laboratory. We would also like to thank the media room staff in the Department of Microbiology and Immunology, University of British Columbia for their assistance in preparing glassware and media used in this study.

REFERENCES

1. **Ackerman, H. W., D. Tremblay, and S. Moineau.** January 2004, posted date. Long-term bacteriophage preservation. World federation for culture collections newsletter. **38:**35-40. [Online.] http://wdcm.nig.ac.jp/wfcc/NEWSLETTER/newsletter38/a4.pdf.

2. **Baines, B.** 2005. Comparison of the effects of microwave irradiation and heat treatment of T4 and T7 bacteriophage. J. Exp. Microbiol. Immunol. **7:**57-61.

3. **Barrett, T. J., H. Lior, J. H. Green, R. Khakhria, J. G. Wells, B. P. Bells, K. D. Greene, J. Lewis, and P. M. Griffin.** 1994. Laboratory investigation of a multistate food-borne outbreak of *Escherichia coli* O157:H7 by using pulsed-field gel electrophoresis and phage typing. J. Clin. Microb. **32:** 3013-3017.

4. **Culkin, K. A., and D. Y. Fung**. 1975. Destruction of *Escherichia coli* and *Salmonella typhimurium* in microwave-cooked soups. J. Milk Food Technol. **38**:8-15.

5. **Goldblith, S. A., and D. I. Wang**. 1967. Effect of microwaves on *Escherichia coli* and *Bacillus subtilis*. Appl. Microbiol. **15**:1371-1375.

6. **Gram, H., H. D. Liebig, A. Hack, E. Niggemann, and W. Ruger.** 1984. A physical map of bacteriophage T4 including the positions of strong promoters and terminators recognized in-vitro. Mol. Gen. Genet. **194**:232-240.

7. **Kakita, Y., N. Kashige, K. Murata, A. Kuroiwa, M. Funatsu, and K. Watanabe.** 1995. Inactivation of *Lacrtobacillus* bacteriophage PL-1 by microwave irradiation. Microbiol Immunol. **39:**571-576.

8. **Kalinski, A., and L. W. Black.** 1986. End structure and mechanism of packaging of bacteriphage T4 DNA. J.Virol. **58**:951-954.

9. **Karam, J. D., and J. W. Drake.** 1994. Molecular biology of bacteriophage T4. ASM Press. Washington, D.C.

10. **Khalil, H., and R**. **Villota.** 1988. Comparative study on injury and recovery of *Staphylococcus aureus* using microwaves and conventional heating. J. Food. Protection. **51**:181-186.

11. **Narasimhan V., and W. Huh.** 1991. Altered restriction patterns of microwave irradiated lamdaphage DNA. Biochem. Int. **25:**363-70.

12. **Park, D., G. Bitton, and R. Melker.** 2006. Microbial inactivation by microwave radiation in the home environment. J. Environ Health. **69:**17-24.

13. **Sagripanti, J., and M. L. Swicord**. 1986. DNA structural changes caused by microwave radiation. Int. J. Radiat. Biol. **50**: 47-50.

14. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning- A laboratory manual. 2nd Ed. 1, pp.2.65-2.81, Cold Spring Harbour Lab Press, New York.

15. **Sasaki, K., Y. Mori, W. Honda, and Y. Miyake**. 1998. Selection of biological indicator for validating microwave heating sterilization. J. Pharm. Sci. Tech. **52:** 313-316.
16. **Travers, A., and G. Muskhelishvili.** 2007. A common topology for bacterial and eukaryotic transcription initiation? EMBO Reports. **8:**147-151.
17. **Vela, G. R., and J. F. Wu.** 1979. Mechanism of lethal action of 2,450-MHz radiation on microorganism. Appl. Environ. Microbiol. **37**:550-55.
18. **Volar, M.** 2005. Optimization of assay conditions in pulsed field gel electrophoresis. J. Exp. Microbiol. Immunol. **7:** 89-93.
19. **Wagner, E. K., and M. J. Hewlett.** 2004. Basic virology, 2nd Ed., p.342-345. Blackwell Science Inc., USA.
20. **Wang, A., N. Cheng, Y. Liou, and K. Lin.** 2001. Inactivation of bacteriophage by microwave irradiation. J. Exp. Microbiol. Immunol. **1:** 9-18.
21. **Webb, B. C., C. J. Thomas, D. W. Harty, and M. D. Wilxocx.** 1998. Effectiveness of two methods of denture sterilization. J. Oral. Rehab. **25:** 416-423.
22. **Yee, J., and R. Marsh.** 1981. Alignment of a restriction map with the genetic map of bacteriophage T4. J. Virol. **1:** 115-124.