Journal of Experimental Microbiology and Immunology Vol. 12:7-13

Membrane Composition as a Factor in Susceptibility of *Escherichia coli* C29 to Thermal and Non-thermal Microwave Radiation

Brad Asay,† Zinaida Tebaykina,† Anastasia Vlasova† and Maggie Wen†
Department of Microbiology and Immunology, University of British Columbia

Bacterial killing can be effectively achieved by microwave irradiation; however, it is unclear if non-thermal effects of microwaves are involved in microbial killing. A number of studies have shown that this effect of microwave radiation is solely due to the heat generated during the experimental process, whereas others state that microbial killing could be achieved through non-thermal microwave exposure. In this study, we showed that non-thermal microwave radiation has no significant impact on viability of *E. coli* C29 bacteria. We also examined the effect of altering membrane composition and fluidity on bacterial resistance to microwave radiation. No change in cell viability was observed upon alteration of membrane composition and fluidity, indicating that they are not significant factors in bacterial resistance to non-thermal microwave radiation. However, our results indicated that membrane fluidity plays an important role in bacterial cell survival during exposure to thermal microwave radiation. We demonstrated that either increasing or decreasing cell membrane fluidity contributed to increased resistance to killing by thermal microwave radiation. The increase in resistance to thermal microwave radiation could also be correlated with decreased permeabilization of cell membrane measured by propidium iodide uptake, suggesting that thermal microwave radiation may inactivate bacteria due to membrane disruption.

Microwave irradiation is an effective way to kill bacteria, and could be adapted as a sterilization technique in the food processing industry (3). Further, microwave irradiation has been successfully utilized in a home setting to disinfect household products contaminated with wastewater, such as sponges and kitchen utensils, and to sterilize some medical devices, such as syringes, where bacterial viability is reduced dramatically following 30 seconds of microwave treatment (13). Despite many studies on microbial killing by microwave radiation, the mechanisms of bacterial killing by microwaves remain poorly understood. It is generally accepted that killing of microorganisms is mainly due to the thermal effect of microwaves (6, 8, 13); however, it is not clear if non-thermal effects of microwave radiation contribute to this process. There are indications that processes other than heating during microwave irradiation might be responsible for bacterial killing. Non-thermal microwave effect has been shown effective for fecal coliform inactivation (7) and killing of microorganisms in a non-thermal continuous-flow microwave generator (8). In this study, we addressed the question of whether non-thermal effects of microwave irradiation reduce bacterial cell viability.

One of the mechanisms that might contribute to bacterial killing by microwaves is increased permeabilization of cell membranes (2, 14). When cells are grown at a certain temperature, they change the lipid content of their membranes in order to maintain the desired level of membrane fluidity at that temperature (4, 10). Therefore, we hypothesized that a change in bacterial membrane composition that affects fluidity would affect bacterial susceptibility to microwave irradiation. To test whether bacterial cells with suboptimal membrane composition are more susceptible to microwaves, we altered the membrane composition of *Escherichia coli* C29 cells, and then examined the effects of non-thermal and thermal microwave radiation on cell viability and permeabilization of both inner and outer membranes.

† These authors contributed equally to this publication.

MATERIALS AND METHODS

Bacterial strains. *E. coli* C29 (*fhu*A22, *fadh*701, *ton*A22, *pho*R19, *omp*F627, *rel*A1, *pit*10, *spo*T1, *rrn*B2, *mcr*B1, *cre*C510, T2r, Hfr (PO2A)) was obtained from the MICB 421 culture collection at the University of British Columbia Department of Microbiology and Immunology. Since *E. coli* C29 strain constitutively expresses alkaline phosphatase and could be induced with IPTG to produce high levels of β-galactosidase, the use of this strain allowed us to easily monitor the release of both enzymes.

Adapting membrane composition and fluidity. Growth temperature were chosen to be 10°C, 26°C, and 37°C. All microwave treatments were performed at 26ºC. Therefore, the cells grown at 26°C had membrane compositions appropriate for that temperature. In contrast, membranes of the cells grown at 37°C had decreased membrane fluidity due to a lower number of double bounds in membrane phospholipid molecules (4, 10). Similarly, cells grown at 10°C adjusted their membrane composition by increasing the number of phospholipid double bonds, making the membranes more fluid (4, 10). These growth temperatures were

chosen because the difference in membrane composition between cells grown at 10ºC and 26ºC is approximately the same as the difference between cells grown at 26ºC and 37ºC (4).

Generation of a log-phase culture. An overnight culture was prepared by inoculating *E. coli* C29 cells from a single colony into 50 mL of Luria-Bertani (LB) broth (10 g/L tryptone, 5 g/L yeast extract, 5g/L NaCl, pH 7.4) into a sterile 125 mL flask, and subsequent incubation in a 37ºC shaking water bath set at 200 rpm for 16 hours. Next morning, the culture was diluted 1/20, and β-galactosidase production was induced by adding isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 280 µg/mL. The culture was subsequently incubated at 10ºC, 26ºC, or 37ºC until a log phase was reached. Cell concentration was estimated by measuring the optical density of the culture at 660 nm in Spectronic 20D+ spectrophotometer (Milton Roy). The culture was presumed to be in a log phase if the optical density reading was between 0.3 to 0.8 OD_{660} units, based on the previously generated *E.coli* growth curve (9).

Cell harvest and preparation of standardized cell suspension. Log-phase cell culture was split into two centrifuge bottles and centrifuged in Beckman J-21 centrifuge at 6000 rpm for 10 minutes. After centrifugation, the cell pellets were resuspended and combined in TM buffer (10 mM Tris, 0.1 mM $MgCl_2$, pH 8.0) to a cell concentration of $2x10^9$ cfu/mL.

Microwave irradiation. 10 mL of cell suspension was transferred into five Petri dishes. The cell suspension was prepared as described in the previous section. The lid from each Petri dish was removed, and the Petri dish was subjected to one of the five following treatments: non-thermal microwave irradiation for 20 or 40 seconds, thermal microwave irradiation for 20 or 40 seconds, or no irradiation (0 seconds), as a control. Non-thermal microwave conditions were maintained to within 1°C of 26°C by placing the Petri dishes in a temperature controlled vessel. This vessel consisted of a large bucket filled partially with ice water and a smaller bucket filled with 26°C water. Petri dishes were first placed in the 26°C water bath. Then, both the water bath and the Petri dish were placed in the ice water bath immediately before microwaving and removed immediately after. The level of ice water in the large bucket was adjusted, so it removed only the excess heat caused by microwaving. Thermal microwave conditions were obtained by placing the Petri dish in the temperature control vessel without either ice or a 26°C water bath. All microwave treatments were performed at a power setting of 5 out of 9 in a microwave (Model ER-754 BTC; Toshiba) with an output power of 650W.

Cell lysis. 3 mL of non-microwaved cell suspension was placed into a 20 mm diameter plastic test tube and kept in an ice water bath. Cells were then sonicated at a power setting of 8 by an ultrasonic homogenizer (Model XL – 2000; Microson) with a 1/8" probe producing an amplitude of 180 microns. Cells were subjected to three 10-second bursts at intervals of 15 seconds.

Viability assay. Following the microwave treatment, serial dilutions were prepared for each sample in TM buffer, and the samples were plated in duplicate to the final plated dilutions of 10^{-1} to 10^{-8} on LB agar plates (prepared based on LB broth with addition of 1.6% agar). The plates were incubated overnight at 37ºC. The next day, the plates were observed for potential contamination, and the formed colonies were manually counted.

Propidium iodide assay. Staining with propidium iodide (PI), a dye that fluoresces upon binding to nucleic acids (15), was used as a measure of cell membrane integrity in this study (4). Microwave-treated cells were diluted in PBS (8 g/L NaCl, 0.2 g/L KCl, 1.44 g/L Na_2HPO_4, 0.24 g/L KH_2PO_4, pH 7.40) to an OD_{660} reading of 0.2 OD_{660} units. Propidium iodide (P-4170; Sigma-Aldrich) was added to each sample to a final concentration of 2.9 µM in duplicate. The samples were then gently vortexed and incubated in the dark for 15 minutes at room temperature. Fluorescence was measured with the Digitlal Filter Fluorometer (Model FM109515; Turner Quantech); the excitation wavelength was set at 490 nm, and the emission wavelength was set at 585 nm.

β-galactosidase and alkaline phosphatase assays. To investigate whether bacterial killing by microwaves could result from disruption of the outer membrane only, or if damage to both the outer and the inner cell membranes was required, we examined

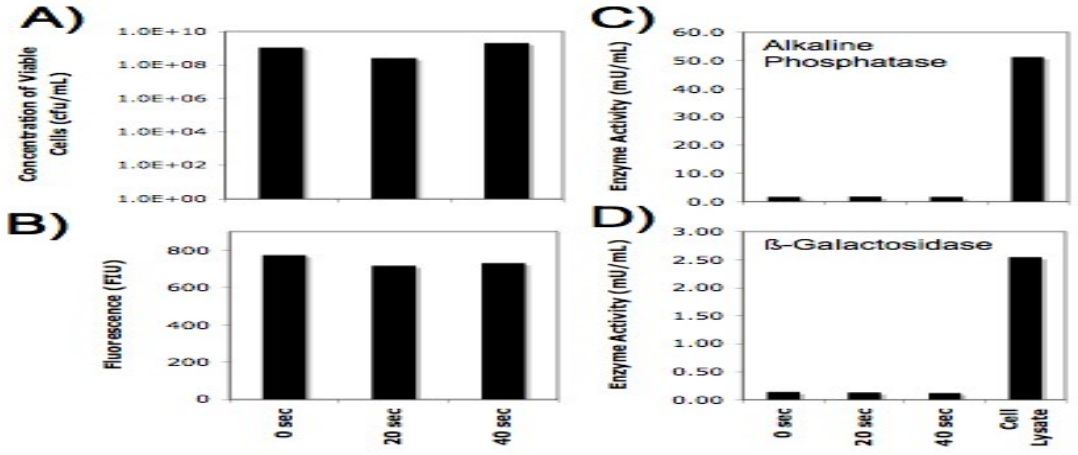

FIG. 1. (A) Since PI uptake is inversely correlated with cell viability, this result was consistent with propidium iodide uptake by the cells, which did not increase after non-thermal microwave exposure (B). A lack of increase in propidium iodide staining and released β- gal (D) also indicated that cell membrane integrity was not adversely affected by the non-thermal microwave treatment. The outer membrane permeability was also shown to be unaffected, since there was no significant change in the amount of periplasmic AP released after the microwave treatment as compared to the non-treated control cells (C). Therefore, non-thermal microwave radiation had no effect on the integrity of both the inner and the outer cell membranes.

the release of a periplasmic (alkaline phosphatase, AP) and a cytoplasmic (β-galactosidase, β-gal) enzymes into the supernatant after microwave treatment. After the microwave treatment, 4 mL of each fraction was centrifuged for 10 min at 8000 rpm in an Eppendorf 5415 microcentrifuge. Supernatant was carefully transferred into a new tube, and the cell pellet was discarded. For the β-galactosidase assay, 1.1 mL of supernatant from each sample was added to a tube containing 0.2 mL of ONPG solution (5 mM in TM buffer) and 0.2 ml of TM buffer warmed up to 30ºC. The tubes were vortexed and then placed back in the 30ºC water bath, and colour development was monitored. Upon noticeable colour formation, the reaction was stopped with 2.0 mL of 0.6 M Na_2CO_3, and the absorbance was measured at 410 nm using Spectronic 20D+ spectrophotometer. The molar extinction coefficient used to convert the absorbance readings into enzyme activity was 15,000 $M^{-1}cm^{-1}$. For the AP assay, 2.0 mL of supernatant from each sample was added to test tubes. 0.1 mL of 2 mg/mL p-nitrophenyl phosphate in TM buffer was added to each test tube and monitored for colour development. When the colour developed, or more than 20 minutes had passed, the reaction was stopped with the addition of 1.0 mL of 5 M NaOH, and the absorbance was measured at 410 nm using Spectronic 20D+ spectrophotometer. The molar extinction coefficient used in the conversion of absorbance and time into enzyme activity was 20,000 M^{-1} cm^{-1}.

RESULTS

Non-thermal effects of microwave radiation do not significantly impact the viability or cell membrane integrity of *E. coli* C29 cells. Microwave irradiation of *E. coli* C29 cells in a temperature-controlled vessel had no effect on cell viability, even when microwave exposure time was increased from 20 to 40 seconds (Fig. 1A). Since PI uptake is inversely correlated with cell viability, this result was consistent with propidium iodide uptake by the cells, which did not increase after non-thermal microwave exposure (Fig. 1B). A lack of increase in propidium iodide staining and released β- gal (Fig. 1D) also indicated that cell membrane integrity was not adversely affected by the non-thermal microwave treatment. The outer membrane permeability was also shown to be unaffected, since there was no significant change in the amount of periplasmic AP released after the microwave treatment as compared to the non-treated control cells (Fig. 1C). Therefore, non-thermal microwave radiation had no effect on the integrity of both the inner and the outer cell membranes.

Changes in cell membrane composition due to growth at different temperatures do not influence the susceptibility of *E.coli* C29 cells to non-thermal microwave radiation. As shown in Figure 2A, the viability of *E.coli* cells did not decrease after the exposure to non-thermal microwave radiation, and this was the case for all three growth temperatures tested (10°C, 26°C and 37°C). This indicated that altering membrane composition by growing cells at higher or lower temperatures did not make cells more susceptible to killing by non-thermal effects of microwave radiation. Moreover, there was no increase in PI uptake or the release of AP and β-gal after the microwave treatment at any of the three growth temperatures, suggesting that varying membrane composition did not make cell membranes more prone to damage by non-thermal microwave radiation (Fig 2 B, C and D). Interestingly, exposure to non-thermal microwave radiation actually caused a

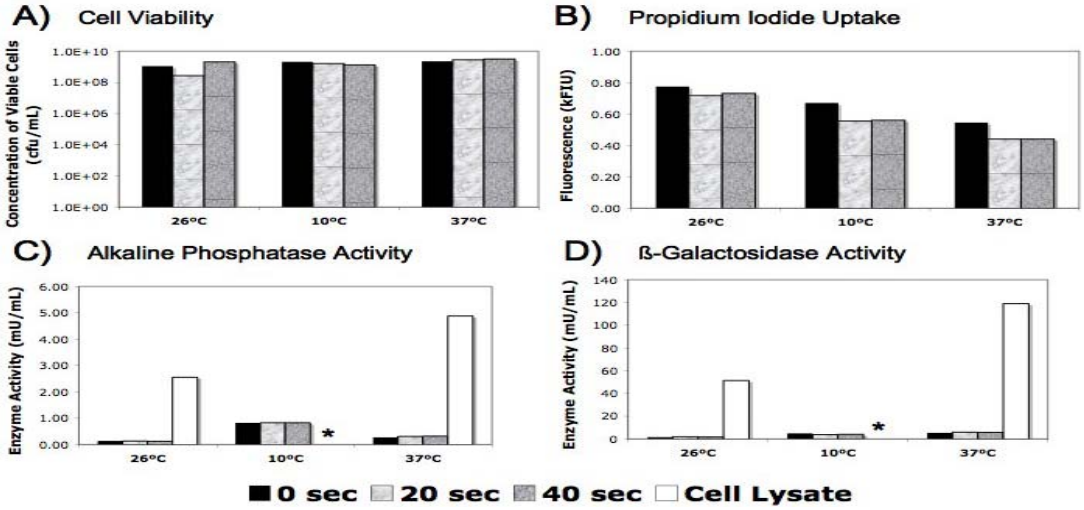

FIG. 2. The Non-thermal effects of 20 and 40 seconds of microwave radiation on *E. coli* C29 cell viability and cell membrane permeability. (A) Cell viability measured by number of colonies formed after duplicate spread plating. (B) Amount of propidium iodide uptake, measured by fluorescence. Cells were diluted in PBS to approximately 0.2 OD_{660} units before staining. (C) Permeability of outer membrane measured by level of alkaline phosphatase activity detected in the supernatant of the cell suspension following centrifugation. (D) Permeability of inner and outer membrane measured by level of β-galactosidase activity detected in the supernatant of the cell suspension following centrifugation. *For (C) and (D) no data was obtained for the enzyme activity of untreated cell lysate for cells grown at 10°C. For (A)-(D) All cells were resuspended in 26°C 0.1% glucose TM buffer before microwaving on setting 5 of 9. Temperature was controlled to remain within 1°C of 26°C during microwave treatment.

slight but consistent decrease in propidium iodide staining relative to the respective controls in all three growth temperature conditions, which did not match our expectations.

Membrane composition affects cell viability after exposure to thermal microwave radiation. Cell viability was decreased by approximately one order of magnitude after 20 seconds of thermal microwave radiation for cells grown at all three temperatures (Fig. 3A). For cells grown at 26°C cell viability was decreased by 8 orders of magnitude after 40 seconds of treatment (Fig. 3A). The decrease in cell viability was less pronounced in the cell cultures grown at 10°C and 37°C with decreases in viability of 7 orders of magnitude and 5 orders of magnitude respectively. This result was consistent with our PI stain, which showed the level of staining after 20 seconds to be 3.8 times the control for the cells grown at 26°C but only 2.7 and 1.8 times the control for the cells grown at 10°C and 37°C respectively (Fig. 3B). PI staining of cells after 40 seconds of treatment showed little variation between cells grown at 26ºC, 10ºC, and 37°C with increases of 4.5, 4.8 and 4.7 times over the control (Fig. 3B).

Membrane composition affects cell membrane permeability after exposure to thermal microwave radiation. Our results show that thermal microwave radiation disrupts the outer membrane of *E. coli* cells and this affect is greatest in cells grown at 26°C (Fig. 3C and 3D). Exposing cells grown at 26°C to 20 seconds of thermal microwave radiation resulted in the release of two thirds of the total AP present, whereas in cells grown at 37°C, only slightly over one third was released after the same treatment (Fig. 3C). For cells grown at 26°C and 37°C, 40 seconds of thermal microwave radiation resulted in an AP activity that was equivalent to that of the untreated cell lysate, which was assumed to contain 100% of the AP present in the cells. No data was obtained for the cell lysate of cells grown at 10°C and therefore we did not have total enzyme content comparisons, however, the trend between the 0, 20, and 40 second treatments appeared similar to that of the cells grown at 26°C. The inner membrane allowed increasing amounts of PI through with increasing length of microwave treatment showing that it was also permeabilized (Fig. 3B). As pointed out above, cells grown at 26°C had the most PI uptake through their

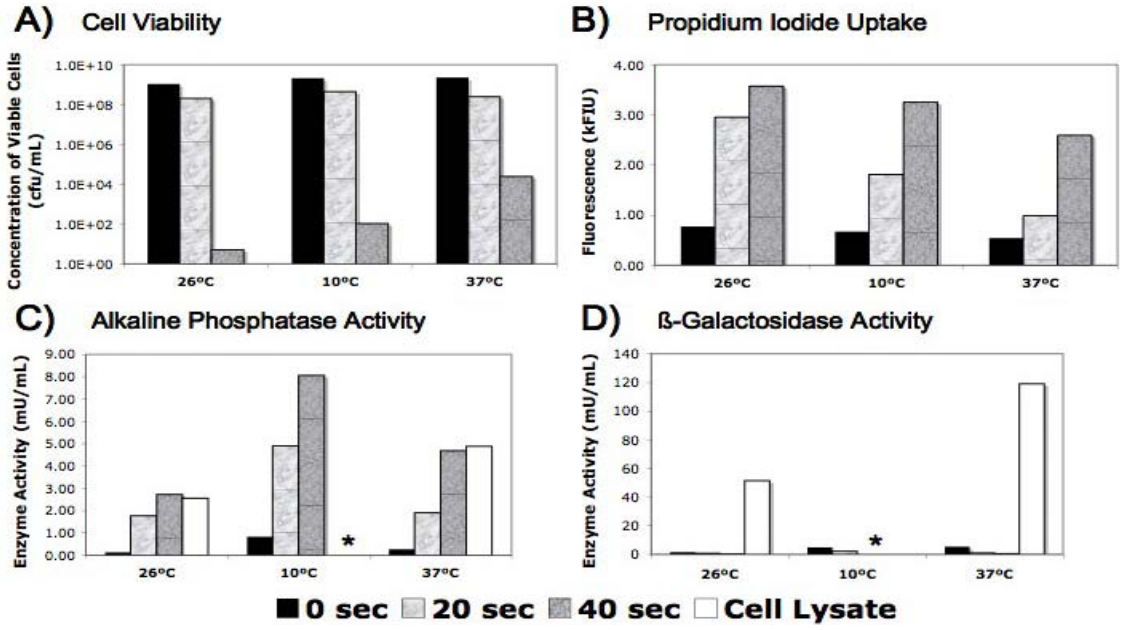

FIG. 3. Effects of Thermal microwave radiation for 20 or 40 seconds on viability and membrane permeability of E. coli C29 cells grown at 10°C, 26°C and 37°C. (A) Cell viability measured by number of colonies formed after duplicate spread plating. (B) Amount of propidium iodide, measured by fluorescence, that permeated both cell membranes and stained nucleic acid. Cells were diluted in PBS to approximately 0.2 OD_{660} units before staining. (C) Permeability of outer membrane measured by level of alkaline phosphatase activity measured in the supernatant of the cell suspension following centrifugation. (D) Permeability of inner and outer membrane measured by level of β-galactosidase activity measured in the supernatant of the cell suspension following centrifugation. *For (C) and (D) no data was obtained for the enzyme activity of untreated cell lysate for cells grown at 10°C. For (A)-(D) All cells were resuspended in 26°C 0.1% glucose TM buffer before microwaving on setting 5 of 9. Temperature was controlled to remain within 1ºC of 26°C during treatment.

cell membranes and cells grown at 37°C had the least after 20 seconds of treatment. This trend showed that cells were less likely to allow PI into their cytoplasm if they were microwaved at a temperature that was different from the one they were grown at, with the effect being greatest for cells that have been cooled rather than warmed. It was difficult to assess permeabilization of the inner membrane using the ß-gal assay, because there was less active β-gal in the supernatant after 20 seconds of thermal microwave treatment than in the control (Fig. 3D) and virtually none after 40 seconds for all three culture conditions, which is a contradictory result to our PI stain.

DISCUSSION

Our results indicated that non-thermal microwave radiation had no impact on *E. coli* C29 viability and did not permeabilize cell membranes. This was not consistent with previous reports (2), which indicated that *E. coli* cell viability decreased with increasing exposure to non-thermal microwave radiation. However, other studies showed that killing of *E. coli* cells via microwave exposure was solely due to thermal effects (6). This contradiction could be explained by how the temperature was controlled during the experiments. In our non-thermal microwave studies, the temperature was kept constant by using a specially designed vessel such that any variation was limited to within 1ºC. However, the previous experiment conducted with *E. coli* C29 (2) did not involve such tight temperature regulation and the temperature actually changed by 9ºC during "non-thermal" microwave treatment.

The major mechanism by which microwaves can affect materials is by rotating polar molecules, a process called dielectric heating. Although non-thermal effects of microwave radiation have been well documented in the field of organic chemistry (9), it is possible that the main biological effects of microwave radiation are caused by the generation of heat. However, this lack of observed effect of non-thermal microwave radiation could also be due to the exposure time being too short. A more significant trend might appear if the exposure time was increased to several minutes.

An unexpected result of non-thermal microwave radiation was the decrease in PI uptake for *E. coli* cells grown at all temperatures. This could mean that non-thermal microwave radiation has an effect on *E. coli* cells other than those tested. Non-thermal microwave radiation has been previously shown to induce a stress response (7, 8). This response may allow cells to remain viable and also to repair their membranes more efficiently. These sorts of effects have previously been shown to occur after heat shock, which also induces the stress response (4, 12). The stress response could also be responsible for the observation that non-treated cells resuspended in media of a different temperature than the incubation temperature showed a decreased PI uptake, as compared to the cells originally grown at the same temperature as the resuspension media. In this case, a rapid temperature change, rather than non-thermal microwave radiation, could induce the stress response.

As expected, thermal microwave radiation caused a decrease in cell viability. Exposing the cell suspension to thermal microwave radiation allowed the temperature to rise by 20°C (to 52°C) after 20 seconds, which has been previously shown to decrease cell viability (11). We then tested if membrane permeability was a factor in the decrease of cell viability. Results from the PI and AP assays indicate that the cell membranes were permeabilized during thermal microwave radiation treatment. We then speculated that the change in membrane composition (due to growth at different temperatures) and membrane fluidity (due to a rapid increase or decrease of temperature) can alter the susceptibility of bacterial cells to thermal microwave radiation. Our results showed that cells which were grown at 37°C experienced less of a decrease in viability after being subjected to thermal microwave radiation. This is expected because cells grown at 37°C have more rigid membranes, which are less susceptible to thermal disruption (5) and may be better suited for higher temperatures. Since we have shown the effects of microwave radiation are only due to the heating of cells, membranes suitable for higher temperature would be advantageous for the cells. Consistent with this explanation, cells grown at 37°C showed less disruption of both cell membranes (Fig. 3B and 3C).

Another explanation for the decrease in viability may be protein denaturation due to the temperature increase. Thermal microwave treatment significantly reduced the activity of β-galactosidase. We speculate that this phenomenon was due to the heat inactivation of the enzyme. It has been shown that many enzymes can be inactivated by high temperatures mainly due to protein denaturation and thus the loss of the proper active site structure (1). However, the activity of AP was not significantly affected by the thermal treatment, as if AP might be more resistant to heat denaturation by microwaves than β-galactosidase.

The viability of cells grown at 10°C did not decrease as much as that of cells grown at 26°C after the thermal microwave treatment. It is unlikely this is due to a change in membrane fluidity because these cells had membranes that were more fluid than cells grown at 26°C, and therefore should be less adapted

to high temperature conditions. It is possible, however, that this was due to a change in membrane composition, particularly, the protein content. It is likely that there were other factors that contributed to an increased resistance to cell death due to microwave irradiation in cells grown at 10°C and 37°C. One of these could be the induction of a stress response which could be triggered by a mild heat and cold shock caused by a change in the surrounding temperature from 10ºC to 26ºC and from 37ºC to 26ºC, respectively. It has previously been demonstrated that stress response of *E. coli* cells makes these cells more resistant to killing (10, 12). The stress response is also able to increase the ability of the cell membranes to reseal (4, 12).

In summary, non-thermal microwave radiation had no significant effect on either viability or membrane permeability of *E. coli* C29 bacteria. Altering membrane composition and fluidity did not make bacterial cells more susceptible to killing by non-thermal microwave radiation. In contrast, membrane fluidity seemed to influence bacterial cell survival during exposure to thermal microwave radiation. Either an increase or a decrease in cell membrane fluidity led to higher resistance to killing by thermal microwave radiation. Decreased permeabilization of cell membranes, as measured by propidium iodide uptake, was also linked to an increase in resistance to thermal microwave radiation. This suggests that thermal microwave radiation may kill bacteria due to membrane disruption.

FUTURE EXPERIMENTS

Since we did not see any significant effects of non-thermal microwave radiation on *E.coli* C29 cells, it would be necessary to confirm whether this was really due to the lack of such effects or whether our microwave time was insufficient. Increasing the microwave exposure time to several minutes would be helpful. Our experimental design also involved adding PI after the microwave treatment. Therefore, it is possible that small holes were formed in the cell membrane but quickly re-sealed after, and consequently this membrane damage was not detected in our assay. For future experiments, one can add PI during the microwave treatment to ensure that all membrane damage is detected. We also saw an unexpected decrease in activity of β-galactosidase after the thermal microwave treatment, suggesting that the enzyme was inactivated by heat. It would help to test the effect of microwave radiaton on an enzyme extract to see if this was indeed true. In addition, it would be more valuable to examine the release, but not necessary the activity of a cytoplasmic protein as a measure of the inner membrane disruption; or use a more thermo stable cytoplasmic enzyme instead of β-galactosidase. It would also be important to test if the temperature change used in our experiment does in fact induce a detectable stress response, which could be examined by measuring the levels of stress-response proteins, such as RpoS (16). It would also be useful to repeat this experiment with an *rpoS*$^-$ mutant in order to assess if these results are in fact due to stress response.

ACKNOWLEDGEMENTS

We are most grateful to Dr. William Ramey and Shaan Gellatly for their valuable suggestions, patience, and dedication by staying long hours in the laboratory to help students. We would also like to thank the media room staff for their reliable autoclaving service. The support for this project was provided by the Department of Microbiology and Immunology at the University of British Columbia.

REFERENCES

1. **Ahern, T. J., and A. M. Klibanov.** 1985. The mechanisms of irreversible enzyme inactivation at 100ºC. Science. **228:**1280-4.
2. **Barnett, C., U. Huerta-Munoz, R. James, and G. Pauls.** 2006. The use of microwave radiation in combination with EDTA as an outer membrane disruption technique to preserve metalloenzyme activity in *Escherichia coli*. J. Exp. Microbiol. Immunol. **9:**1-5.
3. **Brinley, T. A., C. N. Dock, V. D. Truong, P. Coronel, P. Kumar, J. Simunovic, K. P. Sandeep, G. D. Cartwright, K. R. Swartzel, and L. A. Jaykus.** 2007. Feasibility of utilizing bioindicators for testing microbial inactivation in sweetpotato purees processed with a continuous-flow microwave system. J. Food Science. **72:**E235-42.
4. **Cao-Hoang, L., F. Dumont, P. A. Marechal, M. Le-Thanh, and P. Gervais.** 2008. Rates of chilling to 0 degrees C: implications for the survival of microorganisms and relationship with membrane fluidity modifications. Appl. Microbiol. Biotech. **77:**1379-87.
5. **Casadei, M. A., P. Manas, G. Niven, E. Needs, and B. M. Mackey.** 2002. Role of membrane fluidity in pressure resistance of *Escherichia coli* NCTC 8164. Appl. Env. Microbiol. **68:**5965-72.
6. **Goldblith, S. A., and D. I. Wang.** 1967. Effect of microwaves on *Escherichia coli* and *Bacillus subtilis*. Appl. Microbiol. **15:**1371-1375.
7. **Hong, S. M., J. K. Park, and Y. O. Lee.** 2004. Mechanisms of microwave irradiation involved in the destruction of fecal coliforms from biosolids. Water Res. **38:**1615-25.
8. **Kozempel, M. F., B. A. Annous, R. D. Cook, O. J. Scullen, and R. C. Whiting.** 1998. Inactivation of microorganisms with microwaves at reduced temperatures. J. Food Protect. **61:**582-5.
9. **Mamut, A., C. Man, J. Schachter, and T. Yung.** 2007. The relative release of alkaline phosphatase and beta-galactosidase of *E. coli* C29, in exponential and stationary phases, through different lysis methods. J. Exp. Microbiol. Immunol. **11:**14-22.
10. **Mansilla, M. C., L. E. Cybulski, D. Albanesi, and D. de Mendoza.** 2004. Control of membrane lipid fluidity by molecular thermosensors. J. Bacteriol. **186:**6681-8.
11. **Osaili, T. M., C. L. Griffis, E. M. Martin, B. L. Beard, A. E. Keener, and J. A. Marcy.** 2007. Thermal inactivation of *Escherichia coli* O157:H7, *Salmonella*, and *Listeria*

monocytogenes in breaded pork patties. J. Food Science. **72:**M56-61.

12. **Pagan, R., and B. Mackey.** 2000. Relationship between membrane damage and cell death in pressure-treated *Escherichia coli* cells: differences between exponential- and stationary-phase cells and variation among strains. Appl. Env. Microbiol. **66:**2829-34.
13. **Park, D. K., G. Bitton, and R. Melker.** 2006. Microbial inactivation by microwave radiation in the home environment. J. Env. Health. **69:**17-24.
14. **Sajin, G., T. Savopol, E. Kovacs, and I. Miron.** 2005. Effects of 1800 MHz GSM radiation on human monocytes and thrombocytes membrane anisotropy. Conf. Proc. IEEE Eng. Med. Biol. Soc. **7:**7313-6.
15. **Suzuki, T., K. Fujikura, T. Higashiyama, and K. Takata.** 1997. DNA staining for fluorescence and laser confocal microscopy. J. Histochem. Cytochem. **45:**49-53.
16. **White-Ziegler, C. A., S. Um, N. M. Pérez, A. L. Berns, A. J. Malhowski, and S. Young.** 2008. Low temperature (23 degrees C) increases expression of biofilm-, cold-shock- and RpoS-dependent genes in *Escherichia coli* K-12. Microbiol. **154**:148-66.