Differential Damage in Bacterial Cells by Microwave Radiation on the Basis of Cell Wall Structure

IM-SUN WOO, IN-KOO RHEE, AND HEUI-DONG PARK

Department of Food Science and Technology and Department of Agricultural Chemistry, Kyungpook National University, Taegu, Korea

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Microwave radiation in Escherichia coli and Bacillus subtilis cell suspensions resulted in a dramatic reduction of the viable counts as well as increases in the amounts of DNA and protein released from the cells according to the increase of the final temperature of the cell suspensions. However, no significant reduction of cell density was observed in either cell suspension. It is believed that this is due to the fact that most of the bacterial cells inactivated by microwave radiation remained unlysed. Scanning electron microscopy of the microwave-heated cells revealed severe damage on the surface of most E. coli cells, yet there was no significant change observed in the B. subtilis cells. Microwave-injured E. coli cells were easily lysed in the presence of sodium dodecyl sulfate (SDS), yet B. subtilis cells were resistant to SDS.

In recent years, the use of microwave radiation has become popular in the food industry for thawing, drying, and baking foods, as well as for the inactivation of microorganisms in foods (21, 31, 32). In particular, microbial destruction by microwave radiation has great potential in the pasteurization of foods (31). Its short heating and exposure time is less destructive to food than longer conventional heating (15).

There have been many studies on the use of microwaves for the reduction of microorganisms in various foods, including turkey, beef, corn-soy milk, chicken, frozen foods, and potatoes (1, 5, 8, 12, 14, 28, 36). All of these works have led to the conclusion that microwave radiation extends food preservation by reducing microbial cells in food. Microwave heating is known to inactivate many microorganisms, such as Escherichia coli, Streptococcus faecalis, Clostridium perfringens, Staphylococcus aureus, Salmonella, and Listeria spp. (2, 4, 5, 9, 10, 14, 15, 19, 20, 23, 24, 30). Bacterial and mold spores, as well as the bacteriophage PL-1, which is specific to Lactobacillus casei, have also been reported to be sensitive to microwave radiation (20–22).

Despite many studies on microbial destruction by microwave radiation, the mechanism of destruction is not fully understood. It is generally thought that the destruction of microorganisms is mainly due to a thermal effect of microwave exposure (16, 37, 38). However, another argument has also been proposed to explain microbial inactivation by microwaves. Several researchers have attempted to ascertain if such radiation has a nonthermal effect on microorganisms (7, 10, 27, 34). The destruction of microorganisms by microwave at temperatures lower than the thermal destruction point has been observed (11, 13, 22, 24, 27). In particular, microwave-stressed cells of S. aureus exhibited a greater metabolic imbalance than conventionally heated cells (27). Morozov and Petin found that hypertonic solutions (1.0%) of sodium chloride were less effective in protecting cells against heat damage during microwave heating than during thermal heating (29). This study examined the mechanism of microbial cell inactivation by microwave heating along with the differences in the effects on gram-positive and -negative bacteria.

Bacterial culture and microwave treatment. E. coli wild-type strain K-12 (3) was obtained from the Korean Collection for Type Cultures, and Bacillus subtilis KM107 (24) was obtained from the stock culture in our laboratory. E. coli was grown in Luria broth (1% Bacto Tryptone, 0.5% yeast extract, 1% NaCl) (33), and B. subtilis was grown in nutrient medium (0.3% Bacto beef extract, 0.5% Bacto Peptone) (35). The bacteria were cultured in 500 ml of the liquid medium at 37°C for 15 h on a rotary shaker (150 rpm). Cells were harvested by centrifugation and washed twice with a sterile 0.9% NaCl solution. The cell pellets were resuspended in a 0.9% NaCl solution at a cell concentration of 10^9 to 10^10 CFU/ml, which was used for the microwave radiation.

For the microwave heating, a 2,450-MHz microwave oven (MR301M; LG Electronics, Inc., Changwoon, Korea) was used. The cell suspensions were divided into 500-ml plastic beakers and maintained at 20°C. The plastic beakers with the cell suspensions were placed individually in the center of the oven and exposed to microwaves at full power (600 W). The temperature changes in the suspensions were monitored with a fluoroptic thermometer (950 channels; Luxtron Co., Santa Clara, Calif.). After microwave radiation, the suspensions were stored at 4°C for the following experiments. Figure 1 shows the correlation between the microwave radiation time and the temperature changes in the bacterial cell suspensions. A linear increase in the temperature relative to exposure time was observed, which was consistent when the microwave radiation was repeated.

Measurements of viable cell counts and nucleic acid and protein amounts. The microwave-radiated cell suspensions were serially diluted with a sterile 0.9% NaCl solution and spread on Luria-Bertani agar (E. coli) or nutrient agar (B. subtilis) plates. The plates were incubated at 37°C for 24 h, and cells were enumerated. Cell density was measured at 600 nm using a spectrophotometer (CE393; Cecil Instruments, Cambridge, United Kingdom). The amount of protein released from the microwave-treated cells was measured at 595 nm by the method of Bradford (6). Bovine serum albumin was used as the standard protein. The nucleic acid content of the supernatants was directly measured at 260 nm using a UV spectropho-
to microwave radiation at 600 W, and its temperature changes were monitored. Microwave exposure time. A bacterial cell suspension in 0.9% NaCl was exposed to microwave radiation. The temperature of 20°C shown on the

vestigated using cell suspensions (10⁹ to 10¹⁰ CFU/ml) of

polymerized samples were sliced with an ultramicrotome and

mission electron microscopy, dehydrated cells were embedded

microscopy. The cells were fixed at 24°C for 60 min with 2.5%

wave radiation, the shape of the cells was examined by electron

Electron microscopy. After the cells were treated by micro-

wave processing. The amount of nucleic acid released into the cell suspension

greatest reduction ratio in the viable counts was observed when the temperature was increased from 50 to 60°C, which was a ca. 3-log reduction in E. coli organisms (from 1.1 × 10⁵ to 2.5 × 10² CFU/ml) and a ca. 2-log reduction in B. subtilis organisms (from 3.3 × 10⁶ to 1.6 × 10⁴ CFU/ml). When the microwave heating temperature exceeded 60°C, the amount by which viable counts were reduced dramatically decreased. When the temperature was increased from to 60 to 80°C, the viable counts were reduced only by factors of 10 in the E. coli and 3 in the B. subtilis cell suspensions. Therefore, it is assumed that microwave heating for microbial inactivation is highly efficient up to a temperature of 60°C, yet not as effective at higher temperatures.

Although E. coli cells were slightly more sensitive to micro-

wave radiation than B. subtilis cells when the temperature was increased from 50 to 60°C, B. subtilis cells were more sensitive than E. coli cells when the temperature was increased from 40 to 50°C. A temperature increase from 40 to 60°C resulted in a ca. 3.23-log reduction in the E. coli and a ca. 3.66-log reduction in the B. subtilis viable cell counts, indicating that B. subtilis cells are more inactivated by microwave heating than E. coli in this temperature shift. Because the cell wall of gram-positive bacteria is generally much thicker and stronger than that of gram-negative bacteria, it was expected that B. subtilis would be more resistant to microwave radiation than E. coli. However, B. subtilis was found to be more sensitive than E. coli when the temperature was increased from 40 to 60°C.

Interestingly, it was observed that cell density in both cell suspensions did not decrease in spite of a significant reduction in the viable counts. This may be due to the fact that the microwave-treated cells were not completely lysed even when they were inactivated by microwave radiation, and thus the cell density did not decrease.

Leakage of cell materials caused by microwave processing. Another general indication of heat damage to microorganisms is the leakage of nucleic acid and protein from cells. Microwave-injured cells have often been reported to release ninhydrin-positive material, purines, and pyrimidines into a suspension (23). Nucleic acid and its related compounds, such as pyrimidines and purines, are well known to absorb UV light at a wavelength of 260 nm. The presence of these materials in a suspension indicates damage to the cell at the membrane level. Furthermore, similarly injured cells are also known to release intracellular proteins into a suspension.

The amount of nucleic acid released into the cell suspension
was analyzed by measuring the absorbance at 260 nm (Fig. 3A). The two bacterial strains showed similar patterns in their release of nucleic acid. The amount of leaked nucleic acid from the cells grew relative to an increase in the microwave-heated temperature of the cell suspension. However, the leakage of nucleic acid from *B. subtilis* was higher than that from *E. coli*. This result would seem to imply that *B. subtilis* suffered greater membrane damage than *E. coli*. The amount of protein released into the cell suspension was also analyzed in both strains (Fig. 3B). Microwave heating up to 40°C resulted in no significant differences in the amount of protein leaked from the cells. However, when the treatment temperature exceeded 40°C, substantial differences in the amount of leaked protein were observed. These results indicate that most of the microwave-heated cells were ghost cells from which intracellular materials were released into the cell suspension. The protein release pattern of the two bacterial strains was the reverse of the nucleic acid release pattern; the amount of leaked protein in *B. subtilis* was found to be much lower than that in *E. coli*. In particular, a low level of protein leakage was observed when *B. subtilis* cells were heated to 60°C, a temperature observed to be sufficient for a 5-log reduction in the viable count. The reason for this is still unknown.

**Effect of microwave radiation on the surface structure of bacterial cells.** The opposite release patterns for the release of nucleic acid and protein in two bacterial strains prompted us to examine the surface structure of microwave-radiated cells (Fig. 4). The untreated cells and cells heated up to 70°C were examined using a scanning electron microscope, and the shapes of their surface structures were compared. It was found that untreated *E. coli* cells had a smooth surface, while most of the microwave-radiated cells exhibited severe destruction. The surfaces of the microwave-heated cells were damaged and had become rough and swollen. However, all the *B. subtilis* cells exhibited the same smooth surface. Whether the cells were microwave heated or not, no damage to their surface structures was observed. This result suggests that the microwave-radiated cells remained unlysed in suspension, although they were inactivated by the radiation. Furthermore, the damage to the surface structure of *E. coli* cells may not, therefore, be the main reason for inactivation by microwave heating.

**Sensitivity of microwave-heated cells to SDS.** In order to investigate the sensitivity of microwave-injured cells to lysis by sodium dodecyl sulfate (SDS), microwave-heated cells in 0.9% NaCl were incubated at 37°C with shaking (150 rpm) in the presence of 0.1% SDS, and the cell density was monitored at 600 nm (Fig. 5). For *E. coli*, the density of the microwave-heated cell suspension was dramatically reduced within an hour of incubation in the presence of SDS, but it did not decrease significantly in the absence of SDS. In the case of the untreated cell suspension, no significant reduction in the cell density was observed during 4 h of incubation, regardless of the presence of SDS. These results support the conclusion that most of the cells inactivated by microwave radiation remain unlysed in a cell suspension in the absence of SDS. In addition, they are also highly sensitive to lysis by SDS.

When the experiment was repeated using microwave-heated *B. subtilis* cells, different results were obtained. In the absence of SDS, the cell density in both the untreated and microwave-heated cell suspensions slightly decreased in a similar pattern. Unexpectedly, however, the cell density in both cell suspensions slightly increased in the presence of SDS. Why cell density increased is still unknown. Although ambiguous results were obtained for the reaction of *B. subtilis* cells to SDS, it was
obvious that microwave-heated \( B. \ subtilis \) cells were not affected by SDS. It was predicted that microwave-injured \( E. \ coli \) cells would be sensitive to SDS and that untreated cells would be resistant. However, in the case of \( B. \ subtilis \), both untreated and microwave-heated cells were unexpectedly resistant to SDS. This may be due to the fact that the cells of \( B. \ subtilis \), a gram-positive bacterium, were not lysed even in the presence of SDS because of their thick and rigid cell wall structure. Effect of microwave radiation on the intracellular components of cells. To investigate the effect of microwave heating on intracellular components, cells that were microwave heated up to \( 70^\circ C \) were examined using a transmission electron microscope (Fig. 6). When microwave heated, both types of bacteria showed several dark spots in their cytoplasm. However, no dark spots were observed in the untreated cells, suggesting that the dark spots were the result of microwave heating. Further studies on the induction of heat shock proteins are in progress to elucidate the mechanism of protein denaturation and aggregation caused by microwave heating. The two bacterial species showed several dark spots in their cytoplasm. However, no dark spots were observed in the untreated cells, suggesting that the dark spots were the result of microwave heating. Heat treatment has been known to cause protein denaturation and aggregation in cytoplasm as well as to induce heat shock proteins. Therefore, the dark spots are thought to be aggregated proteins caused by microwave heating. The two bacterial strains showed similar results for protein aggregation regardless of their cell wall structure, which suggests that protein aggregation may participate somehow in microbial inactivation caused by microwave heating. Further studies on the induction of heat shock proteins are in progress to elucidate whether microwave heating induces heat shock proteins in bacterial cells.

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REFERENCES

