Susceptibility of *Escherichia coli* O157:H7 grown at low temperatures to the krypton-chlorine excilamp

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This study was conducted to investigate the resistance of *Escherichia coli* O157:H7 to 222-nm krypton-chlorine (KrCl) excilamp and 254-nm low-pressure Hg lamp (LP lamp) treatment according to growth temperature. As growth temperature decreased, lag time of *E. coli* O157:H7 significantly increased while the growth rate significantly decreased. Regardless of growth temperature, the KrCl excilamp showed higher disinfection capacity compared to the LP lamp at stationary growth phase. KrCl excilamp treatment showed significantly higher reduction as growth temperature decreased. Conversely, reduction levels according to growth temperature were not significantly different when the pathogen was subjected to LP lamp treatment. Inactivation mechanisms were evaluated by the thiobarbituric acid reactive substances (TBARS) assay and SYBR green assay, and we confirmed that lipid oxidation capacity following KrCl excilamp treatment increased as growth temperature decreased, which was significantly higher than that of LP lamp treated samples regardless of growth temperature. DNA damage level was significantly higher for LP Hg lamp treated samples compared to those subjected to the KrCl excilamp, but no significant difference pursuant to growth temperature was observed. At the transcriptional level, gene expression related to several metabolic pathways was significantly higher for the pathogen grown at 15°C compared that of 37°C, enabling it to adapt and survive at low temperature, and membrane lipid composition became altered to ensure membrane fluidity. Consequently, resistance of *E. coli* O157:H7 to the KrCl excilamp decreased as growth temperature decreased because the ratio of unsaturated fatty acid composition increased at low growth temperature resulting in higher lipid oxidation levels. These results indicate that KrCl excilamp treatment should be determined carefully considering the growth temperature of *E. coli* O157:H7.

*Escherichia coli* O157:H7 has been associated with 350 outbreaks over a twenty year period¹. This pathogen has caused severe public health and economic problems worldwide with numerous high profile outbreaks (CDC). One of the biggest risks of *E. coli* O157:H7 is related to its low infective doses². Ingestion of as few as 10 organisms may be sufficient to cause infection, which results in bloody diarrhea and hemolytic uremic syndrome³. Because cross-contamination during processing commonly occurs from utensils and surfaces⁴, even slight contamination of the pathogens onto surfaces or work areas may cause serious outbreaks⁴. For example, stainless steel, which is still widely used for constructing processing equipment, can be contaminated with the pathogen. Several research investigations reported that *E. coli* O157:H7 can survive on stainless steel for an extended time (28–60 days) at low temperatures⁵,⁶. These results indicate that the pathogen can survive on stainless steel at numerous temperatures and result in cross contamination from stainless steel to food or medical equipment. Through the present time, sodium hypochlorite (NaOCl) has been used widely to prevent outbreaks and sanitize stainless steel in numerous facilities. However, several disadvantages of NaOCl have been reported including, corrosion of a variety of substances⁷.

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In recent years, several research studies have reported on the inactivation of *E. coli* O157:H7 on stainless steel using ultraviolet-C as an alternative to NaOCl. Ultraviolet-C is one of the non-thermal technologies approved by the U.S. Food and Drug Administration, and widely used to sterilize food surfaces, equipment as well as water. Ultraviolet-C ranges 200 to 280 nm, and it is widely known that 254 nm is the most effective wavelength for disinfecting bacteria within this range. When DNA absorbs UV light at 254 nm, nucleotide base pairing is altered, thereby creating new linkages on DNA strands. As a result, DNA replication is blocked which eventually leads to cell death. Therefore, low pressure mercury lamps (LP lamp) have been used widely to provide monochromatic 254 nm irradiation for the food and clinical industry. Even though LP lamps are cheap and energy efficient, they have critical drawbacks. Their disadvantages include a long warm-up time, a short life time and risk of mercury leakage. In particular, mercury leakage from LP lamps can cause severe human health hazards as well as environmental damage. Moreover, a new international binding treaty instrument called the Minamata Convention was approved, which restricts the usage of mercury. For the reasons given above, the necessity of developing a new source of ultraviolet-C irradiation has been emphasized recently. In the last decades, dielectric barrier discharge (DBD)-driven excilamps (excimer or exciplex lamp) have been introduced as an alternative to LP lamps. Excilamps utilize the non-equilibrium radiation of excimer and exciplex molecules, and emit a narrow band wavelength range from 172 to 345 nm. The merits of excilamps compared to LP lamps include absence of mercury, longer life time, wavelength-selective application and so on. Recently, numerous attempts have been made by researchers to show bacterial disinfection by excilamps. G. Matafonova et al. reported that the KrCl excilamp (222 nm) effectively inactivates gram positive and gram negative bacteria in liquid suspensions. Also, inactivation of *E. coli* O157:H7 by excilamp at 222 nm was higher than at 282 nm and at 254 nm at similar levels of UV fluence. Besides in liquid samples, Ha et al. indicated that the KrCl excilamp is more effective than the LP lamp on solid surfaces. In practice, *E. coli* O157:H7 encounters various environments such as high/low temperatures, diverse pH, and antibiotics. To survive and grow, *E. coli* O157:H7 has adapted to various environments via responses that include in DNA supercoiling, regulating production of protein, fatty acid composition, and DNA transcription. Within various environmental parameters, temperature has been recognized as a cardinal factor controlling microbial population. A great deal of inquiry has been directed toward high/low temperature adaptation of *E. coli* O157:H7. However, a large number of studies have been made at optimum or higher temperatures and focused on the virulence factor, stress response, and gene expression. Jenkins et al. found that stationary phase cells are extremely resistant to various stresses and inimical processes because the rpoS gene is expressed at stationary phase, which is associated with many stress resistance gene such as appR (acid phosphatase expression), nur (near UV resistance) and csi (carbon starvation). Therefore, we investigated the resistance of *E. coli* O157:H7 to the LP lamp or KrCl excilamp at stationary phase. Final cell numbers at the stationary phase were about 10^10^ CFU/ml regardless of growth temperature.

**Results**

**Growth curves of *E. coli* O157:H7 after growing at different temperatures.** Temperature has a significant effect on the growth of *E. coli* O157:H7 (Fig. 1). Lag time and growth rate were significantly affected by temperature while final cell numbers were not significantly different. Lag time increased as temperature decreased. The mean lag times were 0.9, 2.7, and 11.5 h for *E. coli* O157:H7 grown at 37, 25, and 15°C, respectively. On the other hand, growth rate decreased as temperature decreased. The incubation times for the pathogens to enter stationary phase were 6, 12, and 42 h for 37, 25, and 15°C, respectively. It is widely accepted that stationary phase is the stage most resistant to at various stresses. Jenkins et al. found that stationary phase cells are extremely resistant to various stresses and inimical processes because the rpoS gene is expressed at stationary phase, which is associated with many stress resistance gene such as appR (acid phosphatase expression), nur (near UV resistance) and csi (carbon starvation). Therefore, we investigated the resistance of *E. coli* O157:H7 to the LP lamp or KrCl excilamp at stationary phase. Final cell numbers at the stationary phase were about 10^10^ CFU/ml regardless of growth temperature.

**Reduction of *E. coli* O157:H7.** Growth temperature had a significant effect on the inactivation of *E. coli* O157:H7 by KrCl excilamp treatment while significant differences were not observed in LP lamp treated cells (Table 1). Reduction levels (log CFU/cm^2^) of *E. coli* O157:H7 (mixed culture of ATCC 35150, ATCC 43889, and ATCC 43890) subjected to KrCl excilamp exposure increased as growth temperature decreased at same dose treatment (mJ/cm^2^). When *E. coli* O157:H7, grown at 37, 25, and 15°C were, subjected to the KrCl excilamp at...
Table 1. Reduction (log CFU/cm²) of E. coli O157:H7 (mixed culture of ATCC 35150, ATCC 43889, and ATCC 43890) subjected to LP lamp and KrCl excilamp treatment after growth at 37 °C, 25 °C, and 15 °C.a,b. Mean values ± standard deviation. aValues in the same column for each dose followed by the same uppercase letter are not significantly different (p > 0.05). bValues in the same row followed by the same lowercase letter are not significantly different (p > 0.05).

| Dose (mJ/cm²) | Treatments | SMAC | 37°C | 25°C | 15°C | SPRAB | 37°C | 25°C | 15°C |
|--------------|------------|------|------|------|------|-------|------|------|------|
|              |            |      |      |      |      |       |      |      |      |
| 26.1         | LP lamp    | 0.25 ± 0.08 Aa | 0.23 ± 0.21 Aa | 0.11 ± 0.20 Aa | 0.21 ± 0.03 Aa | 0.24 ± 0.12 Aa | 0.26 ± 0.20 Aa |
|              | KrCl excilamp | 0.79 ± 0.15 Ba | 1.01 ± 0.27 Ba | 1.19 ± 0.50 Ba | 0.81 ± 0.21 Ba | 1.10 ± 0.44 Ba | 1.15 ± 0.18 Ba |
| 69.6         | LP lamp    | 0.53 ± 0.28 Aa | 0.29 ± 0.25 Aa | 0.23 ± 0.28 Aa | 0.26 ± 0.05 Aa | 0.63 ± 0.23 Aa | 0.56 ± 0.28 Aa |
|              | KrCl excilamp | 1.46 ± 0.43 Ba | 1.44 ± 0.48 Ba | 1.83 ± 0.38 Ba | 1.25 ± 0.49 Ba | 1.85 ± 0.83 Ba | 1.74 ± 0.20 Ba |
| 131.3        | LP lamp    | 0.65 ± 0.30 Aa | 0.78 ± 0.24 Aa | 0.76 ± 0.55 Aa | 0.35 ± 0.06 Aa | 0.97 ± 0.14 Aa | 0.88 ± 0.55 Aa |
|              | KrCl excilamp | 1.72 ± 0.38 Ba | 2.47 ± 0.11 Bb | 2.79 ± 0.20 Bb | 1.69 ± 0.19 Bb | 2.32 ± 1.09 Bb | 2.73 ± 0.46 Bb |
| 156.6        | LP lamp    | 0.74 ± 0.21 Aa | 1.15 ± 0.22 Aa | 0.94 ± 0.47 Aa | 0.42 ± 0.07 Aa | 1.31 ± 0.06 Ab | 1.14 ± 0.47 Ab |
|              | KrCl excilamp | 2.48 ± 0.26 Ba | 3.12 ± 0.21 Bb | 4.74 ± 0.33 Bc | 2.17 ± 0.12 Ba | 2.80 ± 0.64 Ba | 4.19 ± 0.32 Bb |

Table 2. Lipid oxidation values (arbitrary fluorescence units, AFU) of E. coli O157:H7 ATCC 35150 subjected to KrCl excilamp and LP lamp treatment after growth at 37 °C, 25 °C and 15 °C.a,b. Mean values ± standard deviation. aValues in the same column followed by the same uppercase letter are not significantly different (p > 0.05). bValues in the same row followed by the same lowercase letter are not significantly different (p > 0.05).

| Dose (mJ/cm²) | Treatments | 37°C | 25°C | 15°C |
|--------------|------------|------|------|------|
|              |            |      |      |      |
| 26.1         | LP lamp    | 0.20 ± 0.59 Aa | 0.38 ± 0.19 Aa | 0.35 ± 0.23 Aa |
|              | KrCl excilamp | 10.74 ± 2.00 Ba | 14.26 ± 0.54 Bb | 28.97 ± 2.11 Bc |
| 69.6         | LP lamp    | 6.61 ± 1.64 Aa | 8.59 ± 1.34 Aa | 8.41 ± 2.03 Aa |
|              | KrCl excilamp | 1.06 ± 0.82 Ba | 1.15 ± 0.68 Ba | 4.64 ± 0.89 Bb |

Table 3. DNA damage values (arbitrary fluorescence units, AFU) of E. coli O157:H7 ATCC 35150 subjected to KrCl excilamp and LP lamp treatment after growth at 37 °C, 25 °C and 15 °C.a,b. Mean values ± standard deviation. aValues in the same column followed by the same uppercase letter are not significantly different (p > 0.05). bValues in the same row followed by the same lowercase letter are not significantly different (p > 0.05).

Lipid oxidation and DNA damage. Growth temperature had a significant effect (p < 0.05) on lipid oxidation and DNA damage values (arbitrary fluorescence units, AFU) of KrCl excilamp-treated E. coli O157:H7 (Tables 2 and 3). The lipid oxidation values were 10.74, 14.26, and 28.97 AFU for 37 °C, 25 °C and 15 °C grown cells, respectively. The DNA damage values were 1.06, 1.15 and 4.64 AFU for 37 °C, 25 °C and 15 °C grown cells, respectively. On the other hand, growth temperature had no significant effect (p > 0.05) on either lipid oxidation or DNA damage of LP lamp-treated E. coli O157:H7. Lipid oxidation values were significantly higher (p < 0.05) for KrCl excilamp-treated cells than for LP lamp-treated cells while DNA damage values were significantly higher (p < 0.05) for LP lamp-treated cells than for KrCl excilamp-treated cells.

Transcriptome analysis of E. coli O157:H7 35150. Transcriptome of E. coli O157:H7 ATCC 35150 grown at 15 °C was compared with that grown at 37 °C. Differentially expressed genes (DEGs) were annotated in the GO database, which is a standardized system for gene functional classification, containing 3 domains that are classified by biological process, cellular components, and molecular functions (Fig. 2). In the category of biological processes, most DEGs were related to the cellular process, metabolic process, and single-organism processes. In the category of cellular components, most DEGs were related to the cell (including cell part) and membrane (including membrane part). Finally in the category of molecular functions, most DEGs were related to binding and catalytic activity. We selected several significant pathways by analyzing up-regulated and down-regulated genes. A variety of genes related to oxidative phosphorylation (Fig. 3A), glycolysis/glucogenesis (Fig. 3B), and citrate cycle (Fig. 3C) were down-regulated. On the other hand, a variety of genes related to fatty acid degradation (Fig. 4A) and flagella assembly (Fig. 4B) were up-regulated.
Membrane fatty acid composition. The ratio of unsaturated fatty acids (USFA) to saturated fatty acids (SFA) was higher for the *E. coli* O157:H7 ATCC 35150 membrane grown at lower rather than higher temperatures (Fig. 5). Relative contents (%) of palmitoleic acid of the cell membrane was 7.2 and 39.3 for *E. coli* O157:H7 ATCC 35150 grown at 37 °C and 15 °C, respectively. On the other hand, relative content of lauric, myristic, and palmitic acids decreased as growth temperature decreased. The decrements (%) were 16.4, 7.8, and 13.2 for lauric, myristic, and palmitic acids, respectively.

Figure 2. Number of differentially expressed genes of *E. coli* O157:H7 35150 for biological process (A), cellular component (B), and molecular function (C) in GO category.

Figure 3. Down-regulated genes (green) in the pathway of oxidative phosphorylation (A), glycolysis/glucogenesis (B), and citrate cycle (C). Pathway analysis of the selected genes was performed using the KEGG database (http://www.genome.ad.jp/kegg).

Figure 4. Up-regulated genes (orange) in the pathway of fatty acid degradation (A) and flagella assembly (B). Pathway analysis of the selected genes was performed using the KEGG database (http://www.genome.ad.jp/kegg).
temperatures. This is why reduction levels by the LP lamp were not significantly different (p-value). Exposure to the LP lamp did not have a significant effect on DNA damage of E. coli O157:H7 subjected to the LP lamp treatment. DNA damage values were significantly larger for LP lamp-treated samples compared to KrCl excilamp-treated samples regardless of growth temperature. Because the amount of DNA did not vary according to growth temperature, DNA damage levels were not significantly different relative to growth temperature. From the results above, we postulate that differing lipid oxidation levels is the major reason for different reduction levels of E. coli O157:H7 grown at different temperatures. Recently, it was found that KrCl excilamp treatment can produce reactive oxygen species resulting in lipid oxidation. However, significant differences were not observed when we quantified protein concentrations of E. coli O157:H7 after growing it at various temperatures. Therefore, protein degradation is not sufficient to describe different reduction levels of E. coli O157:H7 grown at different temperatures. Furthermore, the lipid oxidation levels of E. coli O157:H7 subjected to KrCl excilamp treatment increased as growth temperature decreased. Simultaneously, DNA damage levels were not significantly different relative to growth temperature. From the results above, we postulate that differing lipid oxidation levels is the major reason for different reduction levels of E. coli O157:H7 grown at different temperatures when subjected to KrCl excilamp treatment. In contrast to the KrCl excilamp, reduction of E. coli O157:H7 grown at different temperatures was not significantly different when subjected to the LP lamp. Because the results can be attributed to low reduction levels (<1.5 log), we confirmed that significant differences were not observed at high reduction levels (about 4 log) relative to the growth temperature. The major bactericidal mechanism of LP lamps is known to be DNA damage, and we identified that DNA damage values were significantly larger for LP lamp-treated samples as compared to KrCl excilamp-treated samples regardless of growth temperature. Because the amount of DNA did not vary according to growth temperature, damage to DNA of E. coli O157:H7 subjected to the LP lamp did not differ significantly according to growth temperature. This is why reduction levels by the LP lamp were not significantly different (p-value) relative to growth temperature.

We assumed that transcriptional differences between E. coli O157:H7 grown at different temperatures affected the degree of lipid oxidation during KrCl excilamp exposure. Therefore, transcriptome of E. coli O157:H7 ATCC 35150 grown at 15 °C was compared with that grown at 37 °C. A number of DEGs were observed for E. coli O157:H7 grown at 15 °C compared to that grown at 37 °C. Among biological processes, metabolic and cellular processes have the largest number of DEGs. We could identify the metabolic process of E. coli O157:H7 at stationary phase, by using the KEGG pathway, which represents a network of interacting molecules. In the present study, TSB was used as the bacterial culture medium, which is rich in glucose as the sole carbon source. Generally, E. coli utilizes glucose during exponential growth phase and then acetate, which is produced as a major product during aerobic fermentation, and is used as a carbon source when glucose becomes depleted. When acetate is also used up, amino acids are utilized by E. coli during stationary phase. In the present study, genes related to glucose metabolic pathway such as glycolysis, TCA cycle and oxidative phosphorylation were down-regulated in E. coli O157:H7 grown at 15 °C compared to 37 °C. On the other hand, genes related to fatty acid degradation and the fatty acid metabolic pathway were up-regulated for this pathogen grown at low temperature. Moreover, TesA and TesB, which catalyze long chain fatty acyl CoA such as palmitoyl-CoA, stearoyl-CoA and Oleoyl-CoA to long chain fatty acids, are upregulated by a 2.09 and 1.91 log2 fold change, respectively (Table S1). Additionally, fadL and palmitic acid, respectively, when growth temperature decreased from 37 °C to 15 °C. Growth temperature of E. coli O157:H7 ATCC 35150 had no significant effect (p-value) on the relative content of the rest of the fatty acids.

Discussion

The KrCl excilamp, a new source of UV-C emitter, has been demonstrated to be effective for controlling major foodborne pathogens on solid surfaces. In the present study, we identified that resistance of E. coli O157:H7 to high dose (>131.3 mJ/cm²) KrCl excilamp treatment was significantly different depending on growth temperature. It seems that sufficient dose (>threshold value) is needed to induce the significant difference. Reduction levels (log CFU/cm²) of E. coli O157:H7 on stainless steel subjected to high dose KrCl excilamp was higher for cells grown at lower temperature (Table 1). We assumed that specific bactericidal targets of E. coli O157:H7, such as DNA, proteins, or lipids are affected by growth temperature. At first, we investigated protein photodegradation, which has been revealed as a one of the bactericidal mechanisms of the KrCl excilamp. However, significant differences were not observed when we quantified protein concentrations of E. coli O157:H7 after growing it at various temperatures (data not shown). Therefore, protein degradation is not sufficient to describe different reduction levels of E. coli O157:H7 grown at different temperatures. Recently, it was found that KrCl excilamp treatment can produce reactive oxygen species resulting in lipid oxidation. Gomez et al. found that KrCl excilamp can utilize advanced oxidation processes. Also, lipid oxidation was observed in KrCl excilamp-treated samples, which was significantly higher than that of LP lamp-treated samples. Moreover, lipid oxidation levels of E. coli O157:H7 subjected to KrCl excilamp treatment increased as growth temperature decreased. Simultaneously, DNA damage levels were not significantly different relative to growth temperature. From the results above, we postulate that differing lipid oxidation levels is the major reason for different reduction levels of E. coli O157:H7 grown at different temperatures when subjected to KrCl excilamp treatment. In contrast to the KrCl excilamp, reduction of E. coli O157:H7 grown at different temperatures was not significantly different when subjected to the LP lamp. Because the results can be attributed to low reduction levels (<1.5 log), we confirmed that significant differences were not observed at high reduction levels (about 4 log) relative to the growth temperature (data not shown). The major bactericidal mechanism of LP lamps is known to be DNA damage, and we identified that DNA damage values were significantly larger for LP lamp treated samples as compared to KrCl excilamp treated samples regardless of growth temperature. Because the amount of DNA did not vary according to growth temperature, damage to DNA of E. coli O157:H7 subjected to the LP lamp did not differ significantly according to growth temperature. This is why reduction levels by the LP lamp were not significantly different (p-value) relative to growth temperature.

We assumed that transcriptional differences between E. coli O157:H7 grown at different temperatures affected the degree of lipid oxidation during KrCl excilamp exposure. Therefore, transcriptome of E. coli O157:H7 ATCC 35150 grown at 15 °C was compared with that grown at 37 °C. A number of DEGs were observed for E. coli O157:H7 grown at 15 °C compared to that grown at 37 °C. Among biological processes, metabolic and cellular processes have the largest number of DEGs. We could identify the metabolic process of E. coli O157:H7 at stationary phase, by using the KEGG pathway, which represents a network of interacting molecules. In the present study, TSB was used as the bacterial culture medium, which is rich in glucose as the sole carbon source. Generally, E. coli utilizes glucose during exponential growth phase and then acetate, which is produced as a major product during aerobic fermentation, and is used as a carbon source when glucose becomes depleted. When acetate is also used up, amino acids are utilized by E. coli during stationary phase. In the present study, genes related to glucose metabolic pathway such as glycolysis, TCA cycle and oxidative phosphorylation were down-regulated in E. coli O157:H7 grown at 15 °C compared to 37 °C. On the other hand, genes related to fatty acid degradation and the fatty acid metabolic pathway were up-regulated for this pathogen grown at low temperature. Moreover, TesA and TesB, which catalyze long chain fatty acyl CoA such as palmitoyl-CoA, stearoyl-CoA and Oleoyl-CoA to long chain fatty acids, are upregulated by a 2.09 and 1.91 log2 fold change, respectively (Table S1). Additionally, fadL.
plays a central role for the uptake of exogenous long chain fatty acid at the outer membrane, and fadD encodes an inner membrane long chain fatty acid CoA ligase, up-regulated to a 3.8 and 4.74 log2 fold change, respectively (Table S1). These results indicate that E. coli O157:H7 grown at 15°C to stationary phase obtains energy by utilizing fatty acids as a carbon source. Because fatty acids are in a highly reduced state and less oxygenated than other carbon sources, a high amount of metabolic energy can be produced by fatty acid metabolism. Farewell et al. also found that fatty acid metabolism is an important part of the adaptation to stationary phase. From the results above, we submit that E. coli O157:H7 growing at low temperature needs fatty acid metabolism to survive in an environment of low protein concentration and low reaction rate with substrates compared to the optimal growth temperature.

Cell motility and membrane fluidity of E. coli O157:H7 is also known to be important for adapting to a low temperature environment. We observed that E. coli O157:H7 increases flagellar motility (Fig. 4B) and altered membrane fatty acid composition when grown at low temperature (Fig. 5). Because membrane fatty acid composition changes during lag phase at low temperatures36, transcriptional differences related to fatty acyl synthesis was not observed at stationary phase in the present study. However, we identified that palmitoleic acid content was significantly larger for E. coli O157:H7 grown at 15°C than that grown at 37°C. On the other hand, saturated fatty acid, lauric acid, myristic acid, and palmitic acid content was significantly reduced for E. coliO157:H7 grown at 15°C compared to that grown at 37°C. Because cis-unsaturated bonds make a “kink” into the acyl chain, contributing to molecular packing and molecular motions37, membrane fluidity increases as the ratio of unsaturated to saturated fatty acid increases. Regrading motility and fluidity, as temperature decreased, E. coli O157:H7 coped with active flagellar movement and an increased proportion of unsaturated fatty acyl. However, lipid oxidation reacts more easily as the degree of cis-unsaturated fatty acid increases. Therefore, membrane lipids of E. coli O157:H7 grown at low temperatures oxidized more easily than those grown at 37°C, and consequently were more susceptible to KrCl excilamp treatment.

In conclusion, gene expression of E. coli O157:H7 changed to adapt and survive at low growth temperatures. As a result, membrane lipid composition was altered to ensure membrane fluidity. Because unsaturated fatty acid composition of E. coli O157:H7 grown at low temperature increased, lipids oxidation level increased when E. coli O157:H7 was subjected to KrCl excilamp treatment. Consequently, resistance of E. coli O157:H7 to the KrCl excilamp decreased as growth temperature decreased while the resistance was not significantly different for the LP lamp. Because E. coli O157:H7 is a major foodborne pathogen which causes severe risk to public health and to the economy, adequate treatment is necessary to control this pathogen. The KrCl excilamp is a promising non-thermal treatment to replace conventional LP lamps. However, excessive treatment causes quality degradation of treated samples as well as leads to economic loss. Therefore, adequate treatment time for the KrCl excilamp should be determined considering the growth temperature of E. coli O157:H7.

Material and Methods

Growth conditions. Three strains each of E. coli O157:H7 (ATCC 35150, ATCC 43889, ATCC 43890) were obtained from the bacteria culture collection of Seoul National University (Seoul, South Korea). A single colony cultivated from frozen stocks on tryptic soy agar (TSA; Difco, Becton, Dickinson, Sparks, MD) was inoculated into 5 ml of tryptic soy broth (TSB; Difco, Becton, Dickinson, Sparks, MD), incubated for overnight in a shaking incubator at 37°C and 250 rpm. Optical density at 600 nm of the cell suspension was determined and adjusted to 0.1 (ca. 10^6 CFU/ml) with 50 ml TSB. Approximately equal populations of cell suspension were combined with TSB, and incubated in a shaking incubator at different temperature (15, 25, and 37°C) and 250 rpm.

Growth curves. Growth of E. coli O157:H7 was determined by withdrawing 200 μl of samples at appropriate time intervals. Absorbance of samples was determined with a spectrophotometer (Spectrmax M2e; Molecular Devices, Sunnyvale, CA) at 600 nm (data not shown). Populations of viable cells withdrawn at the adjusted time intervals (based on absorbance data) were enumerated by plating on Sorbitol MacConkey (SMAC) agar (Difco). Plate counts were used to determine early stationary phase, which was 52, 12, and 7 h for 15, 25, and 37°C, respectively.

Cell suspension and inoculation. Cell suspensions, incubated at different temperatures, were collected by centrifugation at 4,000 × g for 20 min at 4°C. The pellets were resuspended in 0.2% PW. The concentration of cells was 10^10 to 10^11 CFU/ml. One-tenth ml of each resuspended cell culture was inoculated by distributing as 10 droplets onto a pieces of stainless steel (2 × 5 cm, No. 4) with a micropipettor. The inoculated stainless steel pieces were dried inside a biosafety hood for 1 h. The final cell concentration was approximately 10^6 to 10^8 CFU/cm².

KrCl excilamp and LP lamp treatment. An excilamp filled with a KrCl gas mixture (KrCl excilamp) and a 254-nm germicidal lamp using low-pressure mercury (LP lamp) was used in this study17. Spectral irradiance of the 222-nm KrCl excilamp was identified in the previous study17. The wavelength gap between the output half-peak-intensity was ca. 2.1 nm. Radiation intensities of the KrCl excilamp and LP lamp were measured using an UV fiber optic spectrometer (Avaspec-ULS2048; Avantes, Eerbeek, Netherlands). Lamp dosages were calculated by multiplying irradiance values by the irradiation times. Inoculated stainless steel was treated at room temperature with the KrCl excilamp or LP lamp at equal dosages of 26.1, 69.6, 113.1, and 156.6 mJ/cm². For microbial enumeration, each treated stainless steel piece immediately transferred into a sterile plastic tube (Corning Science Mexico, Reynosa, Mexico) containing 30 ml of sterile 0.2% peptone water and 3 g glass beads (425–600 μm; Sigma–Aldrich, St. Louis, MO, USA) and homogenized for 2 min using a vortex mixer (WISE Mix VM-10, DAIHAN Scientific Co., Seoul, Korea) at a maximum speed18. After homogenization, 1 ml samples were 10-fold serially diluted with 9 ml of sterile 0.2% peptone water and 0.1 ml of stomached or diluted samples were spread plated onto Sorbitol MacConkey (SMAC) agar (Difco). All plates were incubated at 37°C for 24 h before...
counting colonies characteristic of the pathogens. Phenol red agar base with 1% sorbitol (SPRAB; Difco) was used to recover injured cells of *E. coli* O157:H7. After incubation at 37 °C for 24 h, typical white colonies characteristic of *E. coli* O157:H7 were enumerated. Randomly selected isolates from SPRAB plates were subjected to serological confirmation as *E. coli* O157:H7 (RIM, *E. coli* O157:H7 latex agglutination test; Remel, Lenexa, KS), because SPRAB is not typically used as a selective agar for enumerating *E. coli* O157:H7. The number of microorganisms in the untreated samples was used as control.

**Bactericidal mechanism.** Lipid oxidation values were observed as the method described by Kang and Kang41. The quantity of malondialdehyde (MDA) was analyzed using the OxiSelect TBARS Assay Kit (Cell BioLabs, Inc., San Diego, CA), following the manufacturer’s directions. One milliliter of treated sample was centrifuged at 10,000 × g for 10 min and the supernatant was discarded and the pellet resuspended in Phosphate Buffered Saline (PBS) containing butylated hydroxytoluene (BHT). Each sample was mixed with 100 µl SDS lysis solution and incubated at room temperature for 5 min. Following incubation, each sample was reacted with 250 µl of TBA reagent at 95 °C for 1 h and cooled to room temperature in an ice bath for 5 min. Fluorescence of the sample was measured with a spectrophotometer (Spectramax M2e; Molecular Devices, Sunnyvale, CA) at excitation and emission wavelengths of 540 and 590 nm, respectively. DNA damage values were observed according to the method described by Han et al.25. Following each treatment, samples were incubated with 100 µg/ml lysozyme at 37 °C for 4 h to break the cell envelope. Then, the solutions were incubated with SYBR green I (1:10,000 dilution; Sigma-Aldrich Ltd., Dublin, Ireland) at working concentration (1:1) for 15 min at 37 °C. Fluorescence of the aliquot of each sample was measured with a spectrophotometer (Spectramax M2e; Molecular Devices, Sunnyvale, CA) at excitation and emission wavelengths of 485 and 525 nm, respectively.

**RNA sequencing analysis.** Total RNA of *E. coli* O157:H7 ATCC 35150 grown under different conditions was extracted using the miRNeasy Mini Kit (Qiagen Inc., Hilden, Germany) according to the manufacturer’s protocol. Transcriptome analysis was performed with the pathogen grown to early stationary phase, which was 52, 12, and 7 h for 15, 25, and 37 °C, respectively. The total RNA was treated with Rnase-Free Dnase I (Qiagen Inc) for 20 min at room temperature to remove residual DNA. Namely, mRNA molecules were purified and fragmented from 2 µg of total RNA using oligo (dT) magnetic beads. The fragmented mRNA was synthesized as single-stranded cDNAs through random hexamer priming. By applying this as a template for second strand synthesis, double-stranded cDNA was prepared. After sequential process of end repair, A-tailing and adapter ligation, cDNA libraries were amplified with the Polymerase Chain Reaction (PCR). Quality of these cDNA libraries was evaluated with the Agilent 2100 BioAnalyzer (Agilent, CA, USA). They were quantified with the KAPA library quantification kit (Kapa Biosystems, MA, USA) according to the manufacturer’s library quantification protocol. Following cluster amplification of denatured templates, sequencing was progressed as paired-end (2 × 100 bp) using Illumina HiSeq2500 (Illumina, CA, USA).

**Transcriptome data analysis.** Low quality reads were filtered according to the following criteria: reads containing more than 10% of skipped bases (marked as ‘N’s); reads containing more than 40% of bases whose quality scores were less than 20; and reads of which average quality scores of each read was less than 20. The whole filtering process was performed using the in-house scripts. Filtered reads were mapped to the reference genome related to the species using the aligner STAR v.2.4.0b. Gene expression level was measured with Cufflinks v.2.1.145 using the gene annotation database of the species. The non-coding gene region was excluded from gene expression measurement using the--mask option. To improve the accuracy of the measurement, multi-read-correction and frag-bias-correct options were applied. All other options were set to default values.

For DEG analysis, gene level count data were generated using HTSeq-count v0.6.1p145 with the option “-m intersection-nonempty” and “-r option considering paired-end sequence”. Based on the calculated read count data, DEG were identified using the R package called TCC⁴⁸. The TCC package applies robust normalization strategies to compare tag count data. Normalization factors were calculated using the iterative DEGES/edgeR method. Q-value was calculated based on the P-value using the p.adjust function of the R package with default parameter settings. The DEGs were identified based on a q value threshold less than 0.05 for correcting errors caused by multiple-testing⁴⁵.

The GO database classifies genes according to the three categories of Biological Process (BP), Cellular Component (CC) and Molecular Function (MF) and provides information on the function of genes. To characterize the identified genes from DEG analysis, the GO based trend test was carried out through the Fisher’s exact test⁴⁷. Selected genes of P-values < 0.001 following the test were regarded as statistically significant. For pathway analysis, genes of q-value < 0.01 and at least a two-fold change (FC ≥ 2) were required. Pathway analysis of the selected genes was performed using the KEGG database (http://www.genome.ad.jp/kegg)⁴⁶⁴⁸, and it was permitted to publish the KEGG database. The sequence reads in this study were submitted to the NCBI SRA database under study number SRP150528 (https://www.ncbi.nlm.nih.gov/sra/SRP150528).

**Membrane lipid composition.** Membrane fatty acid profiles of *E. coli* O157:H7 ATCC 35150 grown at different temperatures were analyzed. *E. coli* O157:H7 ATCC 35150 was incubated to early stationary phase and collected by centrifugation at 4,000 × g for 20 min at 4 °C. Each pellet was subjected to fatty acid extraction as described in MIDI Technical note no. 101⁴⁹. Mixtures of hexane and methyl tert-buty1 ether were used to extract the fatty acid methyl esters (FAMEs). FAMEs in the upper phase were analyzed on an Agilent gas chromatograph (model 7890A, Agilent Technologies, Santa Clara, CA, USA) equipped with a split-capillary injector and a flame ionization detector⁵⁰. Separations were obtained using a DB-23 column (60 mm × 0.25 mm I. d., 0.25 um, Agilent Technologies). The injector temperature was set at 250 °C, the column oven at 50 °C for 1 min, followed...
by increasing at a rate of 15 °C/min to 130 °C, 8 °C/min to 170 °C, and 2 °C/min to 215 °C, and holding for 10 min. Hydrogen, air, and helium were used as the carrier gas, and the flow rate was set to 35 mL/min, 350 mL/min, and 35 mL/min, respectively. The detector temperature was held at 280 °C. Supelco 37 component FAME mix (Supelco, Inc., PA, USA) was used for analyzing fatty acid profiles.

**Statistical analysis.** All experiments except transcriptome analysis were replicated three times. Transcriptome analysis were replicated. All data were analyzed by the analysis of variance procedure of the Statistical Analysis System (version 9.3, SAS Institute, Cary, NC) and mean values were separated using Duncan’s multiple-range test. Significant differences in the processing treatments were determined at a significance level of \( p = 0.05 \).

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Author Contributions
Professor Dong-Hyun Kang managed the project. Jae-Ik Lee and Dr. Sang-Soon Kim conducted the experiments, and analyzed the data.

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