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Antibacterial effect and mechanism of high-intensity 405 ± 5 nm light emitting diode on *Bacillus cereus*, *Listeria monocytogenes*, and *Staphylococcus aureus* under refrigerated condition

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ABSTRACT

This study investigated the antibacterial effect of 405 ± 5 nm light emitting diode (LED) on *Bacillus cereus*, *Listeria monocytogenes*, and *Staphylococcus aureus*, and examined its antibacterial mechanism by determining the bacterial membrane and DNA damages. A 405 ± 5 nm LED illuminated the Gram-positive pathogens until 486 J/cm^2 at 4 °C. Weibull model was used to calculate reliable life (t_R) to compare bacterial sensitivities to LED illumination. The membrane damage was determined by NaCl and LIVE/DEAD® assay, while comet assay and DNA ladder analysis were conducted to determine DNA degradation. The illumination resulted in 1.9, 2.1, and 1.0 log reductions for *B. cereus*, *L. monocytogenes*, and *S. aureus* at 486 J/cm^2, respectively. The comparison of t_R values revealed that *L. monocytogenes* was identified as the most susceptible strain to LED illumination. The percentage of the bacterial sensitivity to NaCl remarkably increased in LED-illuminated cells compared to non-illuminated cells. Moreover, loss of membrane integrity was confirmed for LED-illuminated cells by LIVE/DEAD® assay, whereas no DNA breakage was indicated by comet assay and DNA ladder analysis. Thus, these findings suggest that the antibacterial effect of 405 ± 5 nm LED illumination on these pathogens might be due to physical damage to bacterial membrane rather than DNA degradation.

1. Introduction

The concern about the microbial food safety dramatically increased recently since the foods contaminated with pathogenic bacteria can threaten our daily life by causing serious illnesses. In the European Union (EU), a total of 5363 confirmed cases of infection, 5118 hospitalizations, and 41 deaths were caused by foodborne outbreaks in 2012. Besides Gram-negative pathogens such as *Salmonella*, Gram-positive *Bacillus cereus* and *Staphylococcus aureus* have been identified as the major causative agents of the foodborne outbreaks [1]. The Centers for Disease Control and Prevention (CDC) in the United States (US) reported that 48 million Americans get sick every year due to foodborne illnesses caused by pathogenic microorganisms. Among 31 known pathogens, *S. aureus* and *Listeria monocytogenes* are listed as one of the top five pathogens in US responsible mainly for foodborne illnesses and deaths, respectively [2].

Pathogenic bacteria are widely existent in the environment and can survive or/and grow at a variety of temperatures. In particular, survival or growth of these pathogenic bacteria in foods at refrigeration temperature is of great concern regarding the microbial food safety. For example, the outbreaks of *L. monocytogenes*, a psychrotrophic pathogen, have been linked to cold-stored ready-to-eat (RTE) products containing fish, meat, and soft cheese due to the ability to grow at 4 °C [3]. For this reason, cold storage which is one of the most widely used preservation techniques should not be applied alone as a mean to control microbial growth. Therefore, it is necessary to combine another technology as a second hurdle with refrigeration to enhance food safety during storage [4,5].

Inactivation of microorganisms using a light emitting diode (LED) of visible wavelengths has recently attracted the interest of researchers due to its antibacterial effect. Some previous studies demonstrated the potential for the application of blue LED as a light therapy to inactivate medically important bacterial pathogens [6,7]. For instance, Elman et al. [6] reported that the treatment of blue light (405–420 nm) resulted in a reduction of 59–67% of *Propionibacterium acnes* that was the major cause of acne on the face. Maclean et al. [7] also demonstrated that methicillin resistant *S. aureus* (MRSA) isolated from infected burns patients was reduced between 56% and 86% of surface bacterial levels in a hospital room by 405 nm LED illumination. Based on such antibacterial effect of blue light, the Food and Drug Administration (FDA) in US approved several blue light devices for the treatment of inflammatory acne vulgaris [8].

Photodynamic inactivation (PDI) by visible blue light requires oxygen, photosensitizer such as porphyrin compounds, and light within

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a range of 400–430 nm that is the absorption range of common porphyrin molecules [9–11]. Once bacteria are exposed to light energy in the presence of oxygen, the endogenous porphyrin compounds absorb the light and then are excited, resulting in production of reactive oxygen species (ROS) [9,12]. The ROS such as superoxide ions, hydroxyl radicals, hydrogen peroxide, and singlet oxygen may play the crucial role in a cytotoxic effect by reacting with intracellular components such as DNA, proteins, and lipids, resulting in bacterial death [4,13,14].

Although some previous studies have shown the antibacterial effect of LEDs between 405–420 nm on the inactivation of *B. cereus*, *L. monocytogenes*, and *S. aureus* in combination with δ-aminolevulinic acid (ALA) as an exogenous photosensitizer [15,16], little research has been performed to investigate their antibacterial effect on the pathogens without the addition of an exogenous photosensitizer which might be applicable and reflect the real food storage condition. Moreover, it is necessary to elucidate which cellular components are directly affected by ROS for a better understanding of its antibacterial mechanism. Thus, the objective of the present study was to examine the antibacterial effect of 405 ± 5 nm LED on *B. cereus*, *L monocytogenes*, and *S. aureus* without the addition of exogenous photosensitizer and to elucidate its antibacterial mechanism by determining bacterial membrane and DNA damage.

2 . Materials and Methods

2.1. Bacterial Strains and Culture Conditions

B. cereus (ATCC 14579), *L. monocytogenes* (BAA-679), and *S. aureus* (ATCC 6538) were obtained from the American Type Culture Collection (Manassas, VA, USA) and stored at −70 °C. Frozen stock cultures were activated in 10 ml of sterile tryptic soy broth (TBS) (Oxoid, Basingstoke, UK) at 37 °C for 24 h. The working cultures at the stationary phase were prepared by incubating culture in TSB at 37 °C for 24 h with at least two consecutive transfers. One ml of culture was centrifuged at 6000 ×*g* for 10 min at 4 °C and washed two times with sterilized phosphate buffered saline (PBS) (Vivantis Technologies Sdn. Bhd., Malaysia). The resultant pellet was suspended in PBS at an initial concentration of approximately 10^8 CFU/ml and the bacterial suspension was used for LED illumination.

2.2. Light Emitting Diode (LED) Source

High intensity 405 ± 5 nm LED (8 × 8 mm shape) (Shenzhen Getian Opto-Electronics Co., Ltd., China) was used in this study. The irradiance (W/cm^2) of 405 ± 5 nm LED was 18 ± 2 mW/cm^2 measured using a 405 nm radiometer (UHC405, UVATA Ltd., Hong Kong) at the surface of bacterial suspension. The dosage obtained from each bacterial suspension was calculated by the following Eq. (1) [17].

$$E = Pt \tag{1}$$

where E = dose (energy density) in J/cm^2, P = irradiance (power density) in W/cm^2, and t = time in sec.

2.3. LED Illumination System

A 405 ± 5 nm LED was attached to a heat sink and cooling fan to minimize heat transfer from the light source to bacterial suspension. To protect the LED from excessive current, a total of 5 Ω of resistance was used in the circuit by connecting two 10 Ω resistors. An acrylonitrile butadiene styrene (ABS) housing was used into LED system to inhibit the entry of external light during illumination. The LED was placed directly above the bacterial suspension into a sterile glass Petri dish (60 mm diameter) at a distance of 4.5 cm that can cover the whole Petri dish [4]. The temperature of the bacterial suspension was monitored during the illumination using Fluke 5.4 thermocouple thermometer (Everett, Washington, USA).

2.4. Bacterial Inactivation by 405 ± 5 nm Illumination

Ten ml of the bacterial suspension (1.2 cm depth) containing the initial population of approximately 10^8 CFU/ml in a glass Petri dish was placed in the LED illumination system and was illuminated by 405 ± 5 nm LED until 486 J/cm^2 (7.5 h) at the set temperature of 4 ± 1 °C in a temperature controlled incubator (MIR-154, Panasonic Healthcare Co., Ltd., Osaka, Japan). The maximum illumination time of 7.5 h was selected based on a previous study [4,13]. Bacterial suspension was also placed in the incubator without LED illumination (dark condition) as a non-illuminated control. A 0.5 ml of bacterial suspension was withdrawn at regular intervals of 97.2 J/cm^2 (1.5 h) and serially diluted with PBS, if necessary. The diluents were plated onto tryptic soy agar (TSA) (Oxoid) using spiral plating (WASP 2, Don Whitley Scientific Ltd., West Yorkshire, UK), followed by incubation at 37 °C for 24–48 h. The number of surviving colonies was enumerated using an automated colony counting system (Acolyte, Synbiosis, Frederick, MD, USA) and expressed as log CFU/ml.

2.5. Weibull Model for Bacterial Inactivation Kinetics

The bacterial inactivation curves were fitted to the modified Weibull model to compare the susceptibility of the bacterial pathogens to the 405 ± 5 nm LED illumination. The Weibull distribution consists of two parameters of α and β [18–20] and the model was described using the following Eq. (2):

$$log_{10}\left(\frac{N}{N_o}\right) = -\frac{1}{2.303}\left(\frac{t}{a}\right)^{\beta} \tag{2}$$

where t is the exposure time (h) to the 405 nm LED, N is the bacterial count after the LED illumination (CFU/ml), N_0 is initial bacterial count (CFU/ml), and α and β are the scale and shape parameters of the Weibull model. Based on the two parameters (α and β), the reliable life (t_R) was calculated with the following Eq. (3) [18].

$$t_R = a(2.303)^{\frac{1}{\beta}} \tag{3}$$

Origin 9.0 software (OriginLab Co., Northampton, MA, USA) was used to analyze the value of the reliable life (t_R) that indicates time required for a 10-fold reduction of the bacterial population in the Weibull distribution. The t_R value is a similar concept to the D-value for the first-order inactivation kinetics [18].

2.6. Bacterial Sensitivity to NaCl by LED Illumination

In order to determine the damage of bacterial cytoplasmic membrane caused by LED illumination, the bacterial sensitivity to NaCl was evaluated by comparing the difference in the bacterial counts (CFU/ml) grown on TSA (non-selective agar) and TSA supplemented with NaCl (Goodrich Chemical Enterprise, Singapore) as selective agar. Non-illuminated and LED-illuminated cells were plated onto TSA with 4% (w/v) NaCl for *B. cereus* and *L. monocytogenes* or 7% (w/v) NaCl for *S. aureus*. The preliminary experiments showed that thesis concentrations were the maximum non-inhibitory concentration for stationary-phase healthy and intact cells that do not affect the formation of colonies on the plates (data not shown). The number of surviving colonies was enumerated after incubation at 37 °C for 24–48 h, and then the percentage of the bacterial sensitivity was calculated using the following Eq. (4) [4].

$$\text{Sensitivity}(\%) = \left[\left(1 - \frac{\text{Colonies on TSA + NaCl}}{\text{Colonies on TSA}}\right) \times 100\right] \tag{4}$$

2.7. Determination of Bacterial Membrane Integrity

The membrane integrity of non-illuminated and LED-illuminated cells was determined using the LIVE/DEAD® *Bac*Light Viability Kit L-7007 (Molecular Probes, Eugene, OR, USA) that consists of two dyes of green fluorescing SYTO®9 (green fluorescence) and red fluorescing propidium iodide (PI) according to the manufacturer's instructions. Briefly, 1 ml of non-illuminated control or bacterial suspension LED-illuminated to 486 J/cm^2 was added to 3 μl of the dye mixture and then was incubated in the dark for 15 min at room temperature. A 5-μl aliquot of the stained bacterial cell suspension was placed on a microscope slide and a square coverslip was applied to the slide. The slides were promptly investigated using an epifluorescent microscope (Olympus BX51, Melville, NY, USA) equipped with an U-RFL-T mercury lamp, a camera (Olympus DP71), and a set of fluorochrome filters with SYTO®9 (WB, 450–480 nm) and PI (WG, 510–550 nm) at magnification of 400×.

2.8. Determination of DNA Degradation

After LED illumination, bacterial DNA degradation was evaluated using the OxiSelect™ Comet Assay Kit (Cell Biolabs, San Diego, CA, USA) according to the manufacturer's instructions with a slight modification. A 10-μl aliquot of non-illuminated or LED-illuminated bacterial suspension to a total dose of 486 J/cm^2 was blended with 90 μl Comet Agarose, 0.5 mg/ml lysozyme (Sigma-Aldrich), 0.25% N-Lauroylsarcosine sodium salt solution (Sigma-Aldrich), and 5 μg/ml RNAse A solution (Sigma-Aldrich), and then 75 μl of the mixture was placed on a Comet slide. The slides were incubated for 15 min at 4 °C in the dark to solidify the agarose, followed by incubating at 37 °C for 20 min. The slides were immersed in 1X lysis buffer (pH 10) at 4 °C for 1 h in the dark, and then immersed in alkaline solution for 30 min at 4 °C in the dark. Electrophoresis was performed using alkaline electrophoresis buffer for 20 min at 12 V at 100 mA. Thereafter, the slides were rinsed three times using distilled water for 2 min, followed by dehydrating with cold 70% ethanol for 10 min, air-drying, and staining by adding 100 μl per well of Vista Green DNA Dye. The slides were visualized under oil immersion using an epifluorescent microscope (Olympus BX51) equipped with a U-RFL-T mercury lamp, a camera (Olympus DP71), and fluorochrome filter of Vista Green DNA Dye (WB, 450–480 nm) at magnification of 1000×.

For DNA ladder analysis, genomic DNA prepared from non-illuminated or LED-illuminated cells at a total dose of 486 J/cm^2 was extracted and purified using GenElute™ Bacterial Genomic DNA Kit (Sigma-Aldrich) according to manufacturer's instruction. The purified DNA was dissolved in 100 μl of Tris-EDTA (TE) buffer. Two μl of DNA extract was mixed with 2 μl of Tri-Color 6X DNA Loading Dye (1st BASE, Singapore). The mixture was electrophoresed by 1% (w/v) agarose gel including FloroSafe DNA Stain (1st BASE) in Tris-acetate-EDTA (TAE, 40 mM Tris-acetate, 1 mM EDTA, pH 8.0) buffer at 100 V to analyze DNA degradation. The gel was visualized using G:Box EF^2 Fluorescence Imaging System (Syngene, Frederick, MD, USA).

2.9. Statistical Analysis

All mean values were obtained from triplicate independent experiments with duplicate sampling (n = 6). The data were expressed by mean ± standard deviation and were analyzed using one-way analysis of variance (ANOVA) with the IBM SPSS statistical software (version 17.0; SPSS Inc., IBM Co., Armonk, NY, USA), where significant differences were accepted at the 95% confidence interval ($P < 0.05$).

3 . Results and Discussion

Change in temperature of bacterial suspension was monitored during the LED illumination to design a control experiment. The temperature of bacterial suspension rapidly increased to 9–10 °C within 1 h of illumination at set temperature of 4 °C (data not shown). Thus, non-illuminated control experiments were carried out at set temperature of 10 °C to eliminate the temperature effect on the bacterial inactivation by the 405 ± 5 nm LED illumination. The refrigerated condition was chosen in this study to simulate an ideal food storage condition. Another reason is that the antibacterial effect of blue LED was enhanced at lower temperatures rather than at ambient temperature according to our previous study [4].

To evaluate the antibacterial effect of 405 ± 5 nm LED, three Gram-positive foodborne pathogens were exposed to 405 ± 5 nm LED for 7.5 h until 486 J/cm^2 at set temperature of 4 °C. The 405 ± 5 nm LED illumination resulted in 1.9-, 2.1-, and 0.9-log reductions for *B. cereus*, *L. monocytogenes* and *S. aureus*, respectively, at a total dose of 486 J/cm^2 (Fig. 1). On the other hand, no significant ($P > 0.05$) inactivation was observed for non-illuminated cells for 7.5 h at set temperature of 10 °C, regardless of the bacterial strain.

The bacterial susceptibility to LED illumination was compared using the reliable life (t_R), analogues to D-value, calculated with the modified Weibull model based on the α and β parameters (Table 1). The α value (scale parameter) corresponds to the mean of distribution explaining the inactivation times (h) of the microbial population and is generally considered as measure of the bacterial resistance to LED illumination with exposure time, while coefficient β determines the shape of Weibull distribution indicating the influence on the predicted death rate [18–20]. Among the bacterial strains in this study, α value of *L. monocytogenes* was two times less than those of *B. cereus* and *S. aureus*. The result corresponded with the highest inactivation observed for *L. monocytogenes* in Fig. 1. The β value of *L. monocytogenes* was nearby 1, which means that the rate of inactivation was not dependent on the light dose (Table 1). The β values of *B. cereus* and *S. aureus* were larger than 1, indicating a higher accumulated damaging and killing rate of the LED illumination to the cells with an increase in light dose [21]. If $\beta < 1$, high inactivation rate would be observed at lower light dose; however the inactivation rates would gradually decrease with increasing light dose [22]. However, when the β coefficient is not equal to 1, both α and β parameters are necessary to assess the sensitivity of the bacterial strains to 405 ± 5 nm LED illumination, and thus t_R values based on these two parameters were calculated. The t_R values of three bacterial pathogens were significantly ($P < 0.05$) different, revealing that *L. monocytogenes* was identified as the most susceptible pathogen to the 405 ± 5 nm LED illumination, followed by *B. cereus* and *S. aureus*. The different bacterial susceptibility to the LED illumination might be due to the differences in bacterial repair system and defense mechanisms during the oxidative stress [23] as well as the variations in the types and the amounts of endogenous porphyrin compounds generated in the bacterial cells [15,24]. For example, Nitzan et al. [15] reported that two staphylococcal strains produced the higher amounts of coproporphyrin than those of *B. cereus* after exposure to blue light (407–420 nm), which caused high level of inactivation of two staphylococcal strains.

Unlike the present study, Maclean et al. [17] showed that *S. aureus* was inactivated at the highest level of about 5-log reduction during the 405 nm LED illumination at a total dose of 36 J/cm^2. Another study performed by Endarko et al. [10] also reported that 5-log reduction of *L. monocytogenes* was achieved by 405 nm LED illumination at a dose of 185 J/cm^2. Such a large difference in the effectiveness of the 405 nm LED illumination might be due to the design of experiments such as initial population, depth and total volume of bacterial suspension, the distance between LED and bacterial suspension, and the treatment temperature [10,25]. For example, the experimental design of Endarken et al. [10] was employed on a 2-ml volume of bacterial suspension (7 mm depth) with 10^5 CFU/ml of initial population, a 2-cm distance between bacterial suspension and LED as well as stirring bar to agitate the suspension, which can probably maximize its bactericidal effect. However, in this study the LED illumination system was designed to simulate realistic food storage conditions under refrigeration. Therefore,

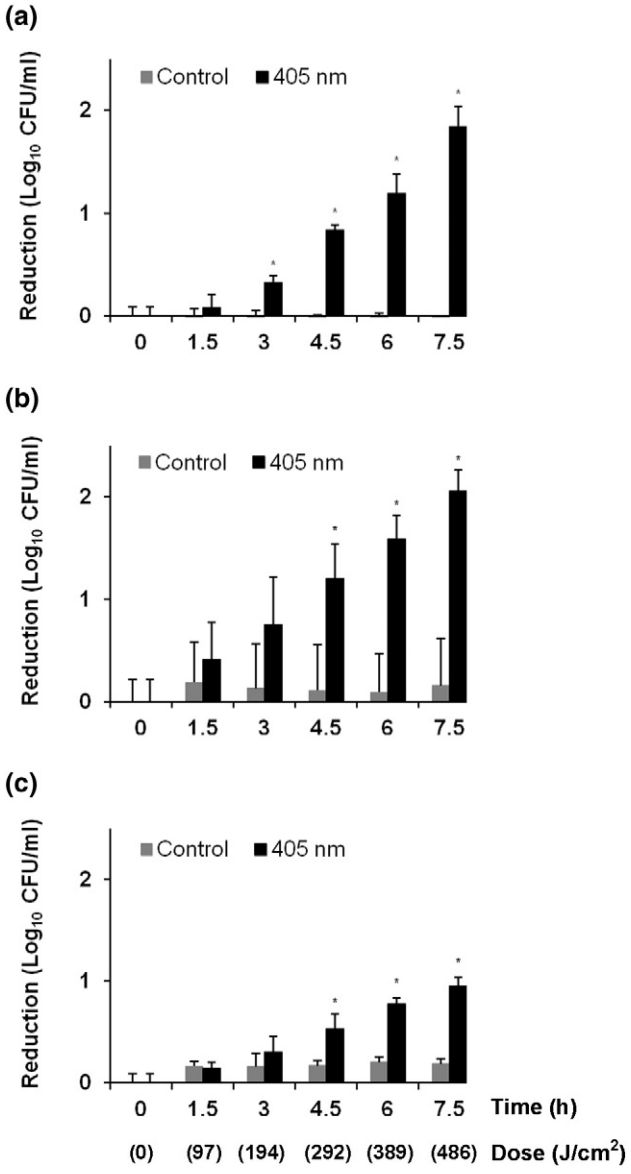

Fig. 1. Antibacterial effect of *B. cereus* (a), *L. monocytogenes* (b) and *S. aureus* (c) during the illumination with 405 ± 5 nm LED at the set temperature of 4 °C. Asterisk (*) indicates significant ($P < 0.05$) difference between LED-illuminated and non-illuminated bacterial cell counts.

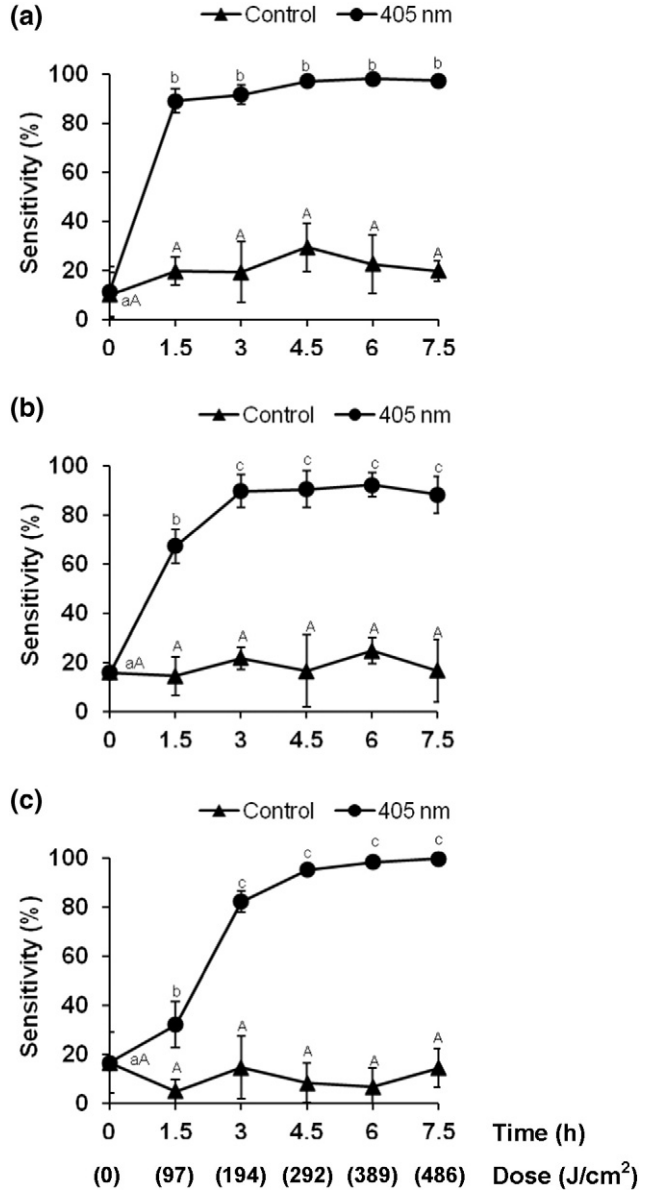

Fig. 2. Percentage of the bacterial sensitivity of *B. cereus* (a), *L. monocytogenes* (b) and *S. aureus* (c) to NaCl (4% for *B. cereus* and *L. monocytogenes*, and 7% for *S. aureus*) during 405 ± 5 nm LED illumination. Different letters within the same curve indicate that the mean values are significantly ($P < 0.05$) different from each other.

higher volume and distance as well as no stirring were applied to avoid agitation of the bacterial suspension under the LED illumination.

The changes in the bacterial sensitivity to NaCl were determined to evaluate whether the LED illumination causes the damage to the bacterial cytoplasmic membrane. In theory, the cells damaged in their membrane would be incapable of recovering on medium containing sublethal concentration of NaCl due to a loss of osmotic functionality of cytoplasmic membranes [26,27]. In this study, the percentage of bacterial sensitivity to NaCl reached more than 90% after 3 h for *B. cereus* and *L. monocytogenes* and after 4.5 h for *S. aureus* (Fig. 2). The maximum percentages were 98.2% for *B. cereus*, 92.3% for *L. monocytogenes*, and

Table 1
Weibull model parameters for the inactivation of *B. cereus*, *L. monocytogenes*, and *S. aureus* by 405 ± 5 nm LED illumination.

Bacterial strain	α (h)			β			t_R (h)	R^2
	Average	95% confidence intervals		Average	95% confidence intervals			
		Lower	Upper		Lower	Upper		
B. cereus	3.35 ± 0.05^b	3.22	3.48	1.75 ± 0.13^b	1.52	2.14	5.30 ± 0.11^b	0.99 ± 0.02
L. monocytogenes	1.49 ± 0.37^a	0.57	2.41	0.95 ± 0.12^a	0.66	1.24	3.57 ± 0.56^a	1.00 ± 0.01
S. aureus	3.63 ± 0.29^b	2.91	4.36	1.11 ± 0.12^a	0.81	1.42	7.72 ± 0.05^c	1.00 ± 0.01

All measurements were done in triplicate with replication, and all values are means ± standard deviation. Different letters within the same column indicate significant ($P < 0.05$) difference.

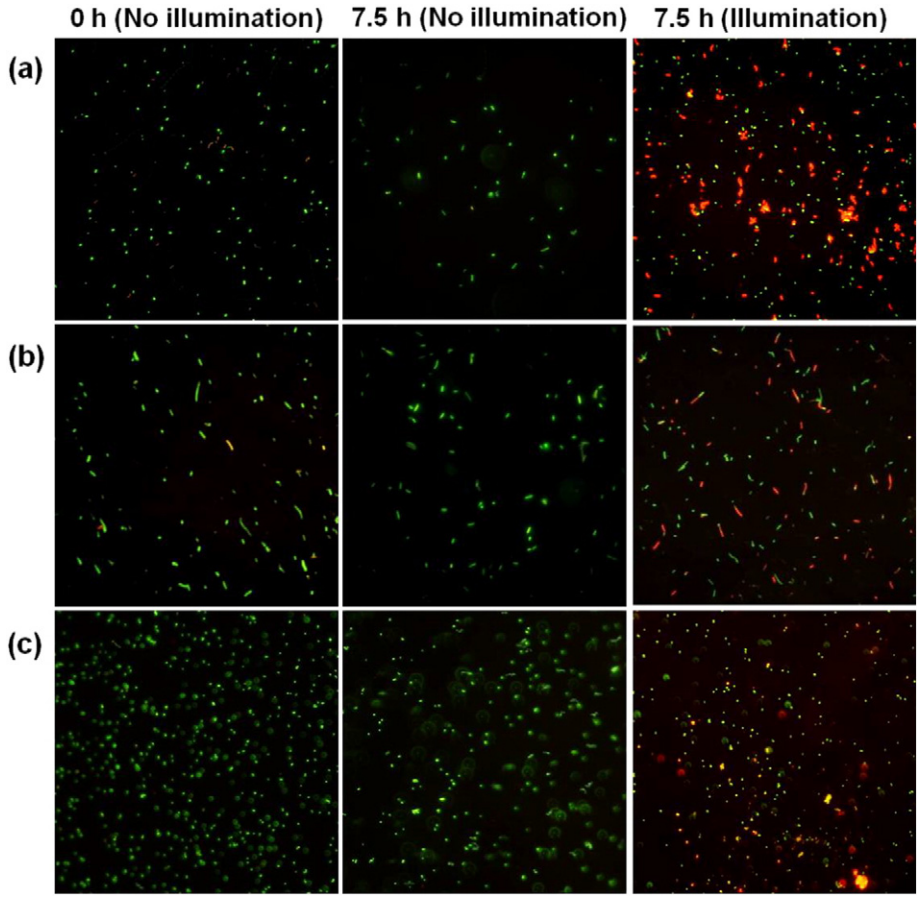

Fig. 3. Epifluorescent micrographs of *B. cereus* (a), *L. monocytogenes* (b) and *S. aureus* (c) cells stained with LIVE/DEAD® *Bac*Light™ before and after LED illumination at a total dose of 486 J/cm^2.

99.6% for *S. aureus* after exposure to 405 ± 5 nm LED for 6–7.5 h. However, no significant ($P > 0.05$) increase in bacterial sensitivity to NaCl was observed in the LED-illuminated cells with the increase in exposure time. These results demonstrate the possibility of damage in cellular membrane by the 405 ± 5 nm LED illumination since bacterial cells become more sensitive to NaCl than the non-illuminated control cells. Similarly, Ghate et al. [4,28] reported that *L. monocytogenes* and *S. aureus* cells in TSB were sensitive to NaCl after treatment of 461 and 521 nm LEDs under the different illumination temperature and pH conditions.

To obtain concrete evidence on cellular membrane damage, the cell membrane permeability of *B. cereus*, *L. monocytogenes*, and *S. aureus* was evaluated using the LIVE/DEAD® *Bac*Light™ assay. The LIVE/DEAD staining of SYTO®9 and propidium iodide (PI) bound to nucleic acid is able to distinguish between damaged and intact bacterial membranes. Green fluorescing SYTO®9 (485/500 nm) penetrates the cytoplasmic membranes of both intact and damaged cells due to low molecular weight (~10 Da), while red fluorescing PI (490/635 nm) of higher molecular weight (668 Da) is only able to enter the damaged cytoplasmic

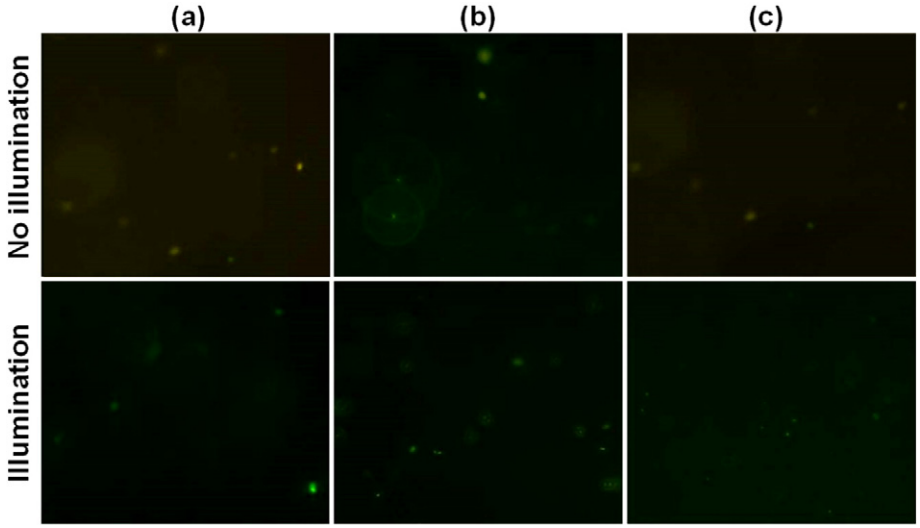

Fig. 4. Comet assay of DNA extracted from healthy, non-illuminated and LED-illuminated *B. cereus* (a), *L. monocytogenes* (b) and *S. aureus* (c) at the dose of 486 J/cm^2.

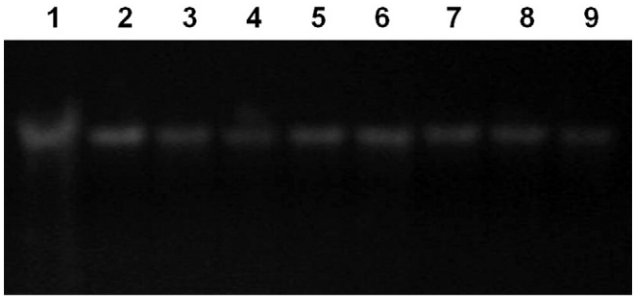

Fig. 5. Agarose gel electrophoresis profiles of DNA extracted from healthy, non-illuminated and LED-illuminated cells at a total dose of 486 J/cm^2. Lane: 1, healthy *S. aureus*; 2, *S. aureus* without LED illumination for 7.5 h; 3, LED-illuminated *S. aureus* for 7.5 h; 4, healthy *L. monocytogenes*; 5, *L. monocytogenes* without LED illumination for 7.5 h; 6, LED-illuminated *L. monocytogenes* for 7.5 h; 7, healthy *B. cereus*; 8, *B. cereus* without LED illumination for 7.5 h; 9, LED-illuminated *B. cereus* for 7.5 h.

membranes, resulting in a reduction in the intensity of SYTO®9 when two stains coexist within the cell [29–31]. In this study, healthy and non-illuminated cells exposed to 10 °C for 7.5 h revealed green fluorescent signal of SYTO®9, whereas some LED-illuminated cells showed red fluorescence (Fig. 3). It is known that there are several mechanisms on the loss of membrane integrity due to the physical functions such as permeability barrier, enzyme activity, membrane potential, and pump activity associated with the membrane [30]. However, the present results strongly suggest that the alteration of bacterial membrane permeability by 405 ± 5 nm LED illumination might be the main reason on the loss of membrane integrity due to the fact that only PI is capable of entering inside the cells with the loss of a permeability barrier [30]. The cellular membrane damage by the LED illumination may be explained with membrane lipids which are one of the major targets of ROS under the oxidative stress condition. ROS generated by the LED illumination may interact directly with unsaturated fatty acids in bacterial membranes and start lipid peroxidation, probably resulting in decreasing their membrane fluidity and then changing membrane components as well as disrupting membrane bound proteins [32]. Similar results were also obtained by Bleicher et al. [29] who reported that the cytoplasmic membrane damage was observed in *Yersinia pestis* and *Burkholderia* strains by metallic copper surfaces due to oxidative damage.

Similarly to cell membrane, genomic DNA is also one of the crucial targets of ROS produced under the oxidative stress conditions such as ionizing radiation and UV light [33,34]. The ROS are known to cause DNA damage by attacking guanine bases and forming oxidized derivatives, such as 8-hydroxy-deoxyguanosine (8-OHdG) [35]. Therefore, the comet assay was carried out to determine DNA degradation by ROS generated from the LED illumination. The comet assay, so called single-cell gel electrophoresis (SCGE), is simple and fast technique to detect the damage such as single- or double-strand breakage in DNA at the individual cell level. The fragments of DNA migrate throughout the electrophoresis and can be visualized by fluorescent microscopy. Microscopic images of the comet assay show a comet with a clear head composed of intact DNA and a tail including degraded fragments of DNA strands [36]. In this study, only clear heads were observed in both non-illuminated and LED-illuminated cells without the presence of tails, regardless of the bacterial strain (Fig. 4).

To confirm the results obtained from the comet assay, DNA ladder analysis was also carried out. Regardless of the bacteria strain, only one band was observed in all the DNA ladder profiles (Lanes 1–9) of healthy, non-illuminated, and LED-illuminated cells (Fig. 5). Moreover, there was no difference in total genomic DNA among healthy, non-illuminated, and LED-illuminated cells. Therefore, the results obtained from comet assay and DNA ladder analysis suggest that the 405 ± 5 nm LED illumination might not cause bacterial DNA breakage. Most likely, the amounts of ROS generated by the LED illumination might not be sufficient to break down DNA strand. The ROS could feasibly target and oxidize other cellular components such as lipids or proteins. Similarly, the study conducted by Nitzan and Ashkenazi [35] demonstrated that various visible light (400–450, 480–550, and 600–700 nm) in the presence of an exogenous photosensitizer resulted in - cytoplasmic membrane damage in *Acinetobacter baumannii* and *Escherichia coli*, while the bacterial DNA was still intact.

4 . Conclusions

This is the first report to investigate the antibacterial effect and mechanism of 405 ± 5 nm LED on the major Gram-positive foodborne pathogens under refrigerated condition. The present results demonstrate that the 405 ± 5 nm LED illumination could reduce the population of *B. cereus*, *L. monocytogenes* and *S. aureus*. Among them, *L. monocytogenes* was found to be the most sensitive pathogen to the LED illumination. The bacterial sensitivity to NaCl was enhanced and the loss of bacterial membrane permeability was determined, while DNA breakage was not observed after the LED illumination. Therefore, these findings suggest that the antibacterial effect of 405 ± 5 nm LED on these Gram-positive pathogens is possibly due to the physical damage to cellular membranes rather than DNA. This study also proposes that 405 ± 5 nm LED in combination with refrigerated conditions might be a promising technology in eliminating these pathogens on foods during storage.

Acknowledgments

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