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Inactivation by 405 ± 5 nm light emitting diode on *Escherichia coli* O157:H7, *Salmonella* Typhimurium, and *Shigella sonnei* under refrigerated condition might be due to the loss of membrane integrity

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ABSTRACT

The objective of this study was to evaluate the antibacterial effect of 405 ± 5 nm light emitting diode (LED) on *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Shigella sonnei*. Its antibacterial mechanism was also investigated by determining the permeability of bacterial membrane and DNA degradation. Bacterial strains in phosphate-buffered saline were exposed to 405 ± 5 nm LED to a final dose of 486 J/cm^2 (7.5 h) at 4 °C. The inactivation curves were fitted by Weibull model to compare the sensitivities of pathogens to the LED illumination by calculating the decimal reduction times (t_R). The bacterial sensitivity to bile salts and NaCl by LED illumination was also determined. LIVE/DEAD® *Bac*Light™ staining as well as comet assay and DNA ladder analysis were carried out to determine the bacterial membrane integrity and DNA degradation, respectively. Results showed that LED illumination inactivated 1.0, 2.0, and 0.8 log CFU/ml for *E. coli* O157:H7, *S.* Typhimurium, and *S. sonnei* for 7.5 h, respectively. The comparison of t_R values demonstrated that *S.* Typhimurium was found to be the most ($P < 0.05$) susceptible strain to LED illumination. Regardless of the bacterial strain, the sensitivity of illuminated bacterial cells to bile salts and NaCl considerably increased compared to non-illuminated controls. Furthermore, LIVE/DEAD® assay clearly showed that LED illumination resulted in loss of bacterial membrane permeability. On the other hand, no DNA degradation was observed by both comet assay and DNA ladder analysis. Therefore, these results suggest that the antibacterial effect of 405 ± 5 nm LED might be partly attributed to the physical damage to bacterial cell membrane. This study proposes that 405 ± 5 nm LED under refrigerated conditions may be effective to control the pathogens on foods.

1. Introduction

Consumption of foods contaminated with infectious levels of bacterial pathogens cause serious illness in humans. According to the data estimated by the United States Centers for Disease Control and Prevention (US CDC), a total of 48 million cases of infections, 127,839 hospitalization 3,037 deaths were caused by major known pathogens and unspecified agents transmitted via food every year in US. The number of infections caused by three major Gram-negative pathogens was as follows: 1 million by *Salmonella*, 131,254 by *Shigella*, 112,752 by non-O157 Shiga toxin-producing *Escherichia coli* (STEC), and 63,153 by O157 STEC (Scallan, Griffin, Angulo, Tauxe, & Hoekstra, 2011; Scallan, Hoekstra, et al., 2011). It was also estimated that annual health related costs due to foodborne disease in US range from $51 billion to $77.7 billion (Scharff, 2012). In Singapore, the Ministry of Health (MOH) estimates that about 0.1 million people per annum seek medical care due to acute diarrheal illnesses (MOH, 2010). Among causative agents, non-typhoidal *Salmonella* spp. have been identified as major pathogenic bacteria to cause foodborne illness, followed by *Campylobacter*, Hepatitis A and E viruses, and *Shigella* spp. during the last decade (Kondakci & Yuk, 2012).

To inhibit or inactivate these pathogenic bacteria on food products during storage, food processors and handlers have

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manipulated intrinsic or extrinsic factors such as temperature, pH, water activity, and antimicrobial agents (Lim, Kim, Lee, & Yuk, 2013; Yuk & Geveke, 2011). Among these, the most widely used preservation technique is cold storage; however, some pathogenic bacteria such as *E. coli* O157:H7 and *Salmonella* spp. are able to survive at refrigeration temperature during transportation and storage. For this reason, refrigeration should not be used as the sole preservation method. Therefore, to ensure food safety and to extend the shelf-life of perishable foods such as ready-to-eat foods, an additional hurdle with refrigeration should be developed and employed for better food preservation without a loss of the food quality (Ghate et al., 2013; Lim et al., 2013).

Ultraviolet (UV) light is able to inactivate microorganisms on the surfaces of foods during storage. However, UV light leads to decolourization in certain products at high dose as well as has harmful effects on the skin tissue and eyes of the operator, resulting in the limitation of UV light as a preservation technology in food industry (Maclean, MacGregor, Anderson, & Woolsey, 2009; Murdoch, Maclean, MacGregor, & Anderson, 2010). To overcome these shortcomings of UV light, light emitting diodes (LED) of visible wavelengths have been investigated as an alternative. A LED, as a semiconductor device, has the capability of emitting visible light within a very narrow wavelength spectrum, resulting in nearly monochromatic light. LEDs have several advantages such as lower energy consumption, high durability, reduced heat output, and long life compared to traditional visible light sources (Ghate et al., 2013; Mori et al., 2007). Also, LEDs can be fabricated in small sizes and various shapes, which could be applied to most designs (Ghate et al., 2013; Mori et al., 2007).

Previous studies have shown that bacteria can be photodynamically inactivated by visible light illumination, especially in the wavelength range of 400–420 nm (Endarko, Maclean, Timoshkin, MacGregor, & Anderson, 2012; Maclean et al., 2009). During photodynamic inactivation (PDI), bacterial cells are exposed to the energy of light, which leads to the excitation of photosensitizers such as either exogenous or endogenous porphyrin molecules. Reactive oxygen species (ROS) are produced, once these porphyrin compounds absorb visible light of 400–420 nm in the presence of oxygen (Lukšiene, 2003, 2005; Lukšiene & Zukauskas, 2009). The ROS such as singlet oxygen, superoxide anion, and the hydroxyl radical may damage membrane lipids, enzymes, proteins, or DNA, consequently inducing bacterial death (Ghate et al., 2013; Lukšiene, 2005; Lukšiene & Zukauskas, 2009).

Recent studies with 405 nm LED have reported its antibacterial effect on many bacterial species, including *Staphylococcus, Streptococcus, Bacillus, Escherichia,* and *Acinetobacter* by the addition of δ-aminolevulinic acid (ALA) as an exogenous photosensitizer (Nitzan, Salmon-Divon, Shporen, & Malik, 2004). For example, methicillin resistant *Staphylococcus aureus* (MRSA) was inactivated by 405 nm LED in clinical environments such as the Burns Unit and the wards (Maclean et al., 2009, 2010). Guffey and Wilborn (2006) reported that the populations of *Pseudmonas aeruginosa* and *Staphylococcus aureus* after 405 nm LED illumination were reduced by 95 and 88%, respectively. Similarly, the study conducted by Murdoch et al. (2010) has shown that *Campylobacter jejuni* and *Salmonella* Enteritidis were inactivated by 405 nm LED illumination without any exogenous photosensitizer. Based on these studies, LED technology has recently received increasing attention in its potential as light therapy for medical purposes, perhaps preventing antibiotic abuse (Maclean et al., 2009). Besides the field of medicine, LEDs have also attracted the attention of researchers in an agriculture area for the control of plant pathogens and to assist in the growth of the plant, and flowering. In particular, blue (430–450 nm) and/or red (650–670 nm) LEDs contribute to the photosynthesis in plants, resulting in the improvement of nutrition quality of vegetables (Olle & Viršile, 2013). However, little information is available on the effectiveness of 405 ± 5 nm LED on the inactivation of various foodborne pathogens and its antibacterial mechanism by endogenous photosensitizer. Therefore, the objective of this study was to investigate the antibacterial effect of 405 ± 5 nm LED on *E. coli* O157:H7, *S.* Typhimurium and *Shigella sonnei*. Its antibacterial mechanism was also elucidated by determining the bacterial sensitivity to bile salts and NaCl as well as by examining loss of bacterial membrane permeability and DNA degradation. Gram-negative pathogens were used in this study since they have similar membrane structure.

2. Materials and methods

2.1. Bacterial strains and culture conditions

E. coli O157:H7 (EDL 933) used in this study was provided by Dr. Henry Mok from the Department of Biological Sciences at National University of Singapore. *Salmonella* Typhimurium (ATCC 14028) and *S. sonnei* (ATCC 29031) were obtained from the American Type Culture Collection (ATCC, 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) for 18–24 h at 37 °C. After two consecutive transfers for 18–24 h at 37 °C, working culture at stationary phase was used for experiments.

2.2. Light emitting diode (LED) source

High intensity 405 ± 5 nm LED was purchased from Shenzhen Getian Opto-Electronics Co., Ltd. (Shenzhen, Guangdong, China). The lamp had a square 8 × 8 mm shape and the irradiance (W/cm^2) of the light emitted from the 405 ± 5 nm LED unit was measured at the surface of bacterial suspension using a 405 ± 5 nm radiometer (UHC405, UVATA Ltd., Hong Kong). The irradiance of the 405 ± 5 nm LED was 18 ± 2 mW/cm^2. The dosage received by each bacterial suspension was calculated using the following equation (Maclean et al., 2009):

$$E = Pt$$

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 LED illumination system has been described elsewhere (Ghate et al., 2013). Briefly, the 405 ± 5 nm LED was attached to a cooling fan and a heat sink to dissipate the heat generated from the LED. A resistance of 5 Ω was used in the circuit in order to protect the LEDs from excessive current. Each LED system was set up in an acrylonitrile butadiene styrene (ABS) housing for the illumination to prevent the entry of external light. The distance between the LED source and the bacterial suspension in a sterile glass Petri dish (60 mm diameter) was adjusted to 4.5 cm to illuminate the entire Petri dish. Fluke 5.4 thermocouple (Everett, WA, USA) was used to monitor the temperature of the bacterial suspension during LED illumination.

2.4. Bacterial inactivation by 405 ± 5 nm LED illumination

One ml of the working culture was centrifuged at 6000 × *g* for 10 min at 4 °C. The obtained pellet was washed with 1 ml of phosphate-buffered saline (PBS; Vivantis Inc., Oceanside, CA, USA) and centrifuged again. The resultant pellet was resuspended in 1 ml of PBS and diluted to a final concentration of approximately