Photochemistry and Photobiology, 2012, 88: 1280–1286

High-Intensity 405 nm Light Inactivation of *Listeria monocytogenes*

Endarko Endarko, Michelle Maclean*, Igor V. Timoshkin, Scott J. MacGregor and John G. Anderson

The Robertson Trust Laboratory for Electronic Sterilisation Technologies (ROLEST), Department of Electronic and Electrical Engineering, University of Strathclyde, Glasgow, UK

Received 6 March 2012, accepted 4 May 2012, DOI: 10.1111/j.1751-1097.2012.01173.x

ABSTRACT

The antimicrobial properties of light is an area of increasing interest. This study investigates the sensitivity of the significant foodborne pathogen *Listeria monocytogenes* to selected wavelengths of visible light. Results demonstrate that exposure to wavelength region 400–450 nm, at sufficiently high dose levels (750 J cm^{-2}), induced complete inactivation of a 5 log$_{10}$ population. Exposure to wavelengths longer than 450 nm did not cause significant inactivation. Analysis of 10 nm bandwidths between 400 and 450 nm confirmed 405(±5) nm light to be most effective for the inactivation of *L. monocytogenes*, with a lesser bactericidal effect also evident at other wavelengths between 400 and 440 nm. Identification of the optimum bactericidal wavelength enabled the comparison of inactivation using 405(±5) nm filtered light and a 405 nm light-emitting diode (LED) array (14 nm FWHM). Results demonstrate similar inactivation kinetics, indicating that the applied dose of 405 nm light is the important factor. Use of the 405 nm LED array for the inactivation of *L. monocytogenes* and other *Listeria* species resulted in similar kinetics, with up to 5 log$_{10}$ reductions with a dose of 185 J cm^{-2}. Comparative data for the 405 nm light inactivation of *L. monocytogenes* and other important foodborne pathogens, *Escherichia coli*, *Salmonella enteritidis* and *Shigella sonnei*, are also presented, with *L. monocytogenes* showing higher susceptibility to inactivation through 405 nm light exposure.

INTRODUCTION

Light inactivation of microorganisms is a research area of increasing interest, not least due to the emergence of resistance to a number of other control methods including the use of disinfectants and antibiotics. For photodynamic inactivation, bacterial exposure to visible-light energy, typically wavelengths in the visible region, causes the excitation of photosensitizer molecules (either exogenous or endogenous), which results in the production of singlet oxygen ($^1O_2$) and other reactive species that react with intracellular components, and consequently induce bacterial cell inactivation (1–5).

Visible light, in combination with a wide range of exogenous photosensitizing molecules such as methylene blue, the cationic thiazine dye toluidine blue, cationic porphyrins, phthalocyanines and chlorins, is well established as being an effective antimicrobial treatment, successfully demonstrated to inactivate bacteria, yeast, fungi, protozoan, parasites and viruses (6–11). Photodynamic inactivation of bacteria through visible-light exposure alone is thought to be caused by the excitation of endogenous photosensitizing molecules present within the bacterial cell, resulting in the production of reactive bactericidal chemical species generated in the same way as occurs with the use of exogenous photosensitizers. Previous studies have indicated that these endogenous photosensitizer molecules are porphyrins (6,12–14).

This study focuses on the sole use of visible light for the inactivation of the bacterium *Listeria monocytogenes*. This is an important Gram-positive bacterial foodborne pathogen, which causes listeriosis, a significant foodborne illness with a high mortality rate in the range of 20–30% (15,16). This study investigates for the first time the sensitivity of *L. monocytogenes* to selected wavelengths of visible-light using a continuous xenon white-light source in combination with a range of optical filters. The finding that within the visible-light spectrum the optimum wavelength for inactivation was 405(±5) nm then enabled a comparison of bacterial inactivation to be made using two different sources of 405-nm light: filtered light from a broadband xenon lamp (405 ± 5 nm) and light from a 405 nm light-emitting diode (LED) light source, with a full-width half-maximum (FWHM) of 14 nm. Comparative data for the 405 nm light inactivation of other *Listeria* species (*L. ivanovii and L. seeligeri*), and also the important foodborne pathogens *Escherichia coli* 0157:H7, *Salmonella enteritidis* and *Shigella sonnei*, are also presented.

MATERIALS AND METHODS

Microorganisms. The microorganisms used in the study were *Listeria monocytogenes* NCTC 11994, *Listeria ivanovii* NCTC 11846, *Listeria seeligeri* NCTC 11856, *Salmonella enterica* NCTC 4444, *Escherichia coli* 0157:H7 NCTC 12900, all obtained from the UK National Collection of Type Cultures, and *Shigella sonnei* LMG 10473, sourced from the Belgian Co-ordinated Collections of Micro-organisms. *Listeria* species are Gram-positive bacteria, and *S. enterica*, *E. coli* and *Sh. sonnei* are Gram-negative bacteria. Microorganisms were cultivated in 100 mL tryptone soya broth (TSB) (Oxoid, UK) at 37°C under rotary conditions (120 rpm). After an 18–24 h incubation period, the cultures were centrifuged for 10 min at 3939 ***g***, and the cell pellet resuspended in 100 mL phosphate-buffered saline (PBS) (Oxoid, UK). The microbial suspensions were then serially diluted in PBS to provide a population density of 10^5 CFU mL^{-1} for experimental use.

Wavelength sensitivity of L. monocytogenes. The wavelength sensitivity of *L. monocytogenes* was determined by exposing bacterial suspensions to various wavelengths of light and assessing the different levels of inactivation achieved. An illustration of the experimental arrangement used to assess the wavelength sensitivity is presented in

*Corresponding author email: michelle.maclean@eee.strath.ac.uk (Michelle Maclean)

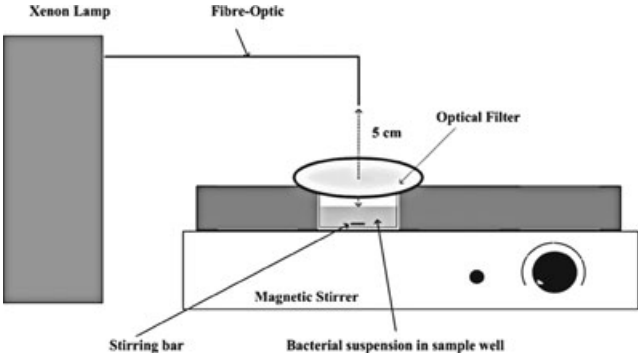

Figure 1. Experimental arrangement for investigation of the wavelength sensitivity of *Listeria monocytogenes*.

Fig. 1. The light source used was a 150 W continuous xenon broadband white-light source (Lightningcure LC5, Hamamatsu Photonics UK, Ltd.), in conjunction with a range of optical filters. For light-treatment, 2 mL volumes of bacterial suspension (7 mm depth) were held in one well of a 12-well multidish (∅ 22 mm) (Nunc, Denmark), with a light shield placed around the well to avoid samples being exposed to unfiltered light. Optical filters were placed on top of the sample well and bacterial suspensions exposed to filtered-light treatment. In order to identify which region of light the bacteria were most sensitive, bacterial suspensions were firstly exposed to broad-bandwidths of light with an irradiance of 126 mW cm^{-2} (measured using a radiant power meter). This was performed using a range of short-wave pass (SWP) and long-wave pass (LWP) filters: 500 nm SWP, 400 nm LWP, 450 nm LWP, and the 500 nm SWP and 400 nm LWP filters combined. Upon identification of the broadband wavelength region to which the bacteria were most sensitive, samples were subsequently exposed to narrow bandwidths of light, with an irradiance of 7–8 mW cm^{-2}, using a selection of narrow bandpass filters (FWHM, 10 nm): 400, 405, 410, 415, 420, 430, 440 and 450 nm.

During exposure, samples were continuously agitated using a magnetic stirring plate in order to ensure uniform suspension of the bacteria. Control samples were also set up which were agitated and left in laboratory lighting conditions. Due to the bacterial suspension having a population density of 10^5 CFU mL^{-1}, and the depth of the suspension being 7 mm, there is no measurable attenuation of the light through the suspension (12). After exposure, test and control samples were immediately plated onto tryptone soya agar (TSA) plates using standard microbiological plating methods, and incubated at 37°C for 24 h before enumeration. Fifty-microliter spiral plate or 100 μL spread plate samples were prepared (WASP 2 spiral plater; Don Whitley Scientific), with each sample being plated in triplicate (at least). If low counts were expected, a larger volume (1 mL) of the test sample was plated for enumeration, thereby providing a detection limit of 1 CFU mL^{-1}.

Exposure to 405 nm high-intensity light. The 405 nm light source used was an LED array with 99 closely packed LEDs in a 11×9 matrix (OptoDiode Corp.), and had an output emission bandwidth of 405 nm (14 nm FWHM). To ensure that any heat produced by the LED array was low and had no effect on the test samples, the array was bonded to a heat sink and fan, and this was mounted in a polyvinyl chloride (PVC) housing designed to include air vents, permitting ventilation around the array and sample dish, preventing a build-up of heat.

For bacterial exposure, a similar experimental set-up was utilized (Fig. 2). Two-mililiter volumes of bacterial suspension were held in the well of a 12-well multidish, with the PVC housing placed on top in order that the LED array was positioned directly above the sample well. Samples were continuously agitated and exposed to increasing durations of 405 nm light, before being plated onto TSA and incubated at 37°C for 24 h. Control samples were set up using the same procedure but exposed to laboratory lighting.

To ensure that light exposure did not induce a bactericidal effect in the PBS, 2 mL volumes of PBS (without bacteria) were exposed to 405 nm light, and bacterial populations added postexposure were monitored and compared with those added to nonexposed PBS controls.

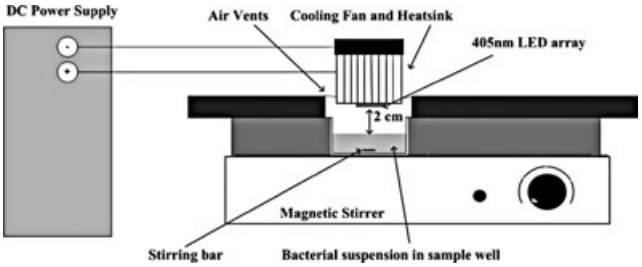

Figure 2. Experimental arrangement for inactivation of *Listeria monocytogenes* using high-intensity 405 nm light from an LED array.

L. monocytogenes was also exposed to 405 nm light in the presence of 100 mM dimethylthiourea (DMTU), a scavenger of reactive oxygen species (ROS), in order to determine whether the presence of an oxygen scavenger inhibited the inactivation process. Exposures were performed following the same method described above.

405 nm dose-dependence experiments. In order to investigate the dependence of the applied dose of 405 nm light on the inactivation of suspensions of *L. monocytogenes*, samples were exposed to four different doses: 61.6, 92.5, 123.3 and 154.1 J cm^{-2}. Samples were exposed to these doses using four different irradiances levels (8.6, 44.7, 66.1 and 85.6 mW cm^{-2}), with the exposure time adjusted in order to maintain the same dose exposure for each experiment, as according to the equation:

$$H = Et \quad (1)$$

where, H is the radiant exposure (dose) in J cm^{-2} or mJ cm^{-2}, E is the irradiance in W cm^{-2} or mW cm^{-2} and t is the exposure time in seconds (17).

Measurement of sample temperatures during light exposure. Sample temperatures were monitored before and after each exposure using a digital thermometer and thermocouple (Kane May 340) with an accuracy of ±0.2%. No significant effects were observed to occur, with the maximum sample temperature recorded as 32°C after the longest exposure time of 144 min. This ensured that the inactivation of *L. monocytogenes*, and the other organisms tested, was a result of visible-light exposure alone and not due to any thermal effects. Consistent population densities of the nonlight-exposed control samples also verified that there was also no lethal temperature change as a result of heat generation by the magnetic stirring plate.

Statistical analysis. In this study, all data were taken with a minimum three replicates for each independent experiment, and the results documented as mean values with standard deviation (SD) being included. Significant differences in experiments were calculated from the data analysis using ANOVA (one way) with 95% confidence interval and $P \leq 0.05$ using MINITAB Release 16.

RESULTS

Visible-light exposure

SWP and LWP filters were selected to assess the wavelength sensitivity of *L. monocytogenes* to inactivation within the range 400–500 nm. Figure 3 shows the effect of different light wavelength ranges on suspensions of *L. monocytogenes*. It can be seen that, for the same applied dose level, the most rapid inactivation rate was found upon exposure to wavelengths of 500 nm and less, with a 5.1 $\log_{10}$ reduction in bacterial population after exposure to a dose of 604.8 J cm^{-2}, and this is likely due to the inclusion of UV wavelengths in the exposing light spectrum. Within the visible-wavelength region tested, *L. monocytogenes* was most susceptible to light of wavelength 400–500 nm, with an approximately 50% faster inactivation rate, at the same applied dose level, than that

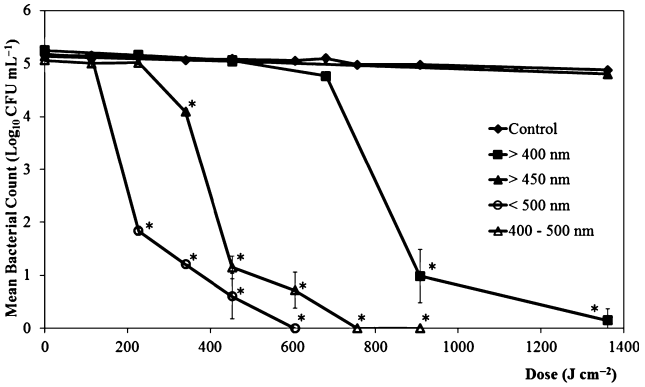

Figure 3. Inactivation of *Listeria monocytogenes* in liquid suspension, upon exposure to differing regions of broadband light using short- and long-wave pass filters. * indicates where a light-exposed bacterial count was significantly different from the nonexposed control count ($P \leq 0.05$ calculated at the 95% confidence interval).

found when exposed to wavelengths of 400 nm and above. It can be observed from Fig. 3 that the applied dose of 1360.8 J cm^{-2} for >450 nm light had almost no effect on the 10^5 CFU mL^{-1} population of *L. monocytogenes*, with only an approximately 0.1 $\log_{10}$ reduction in bacteria cell population being achieved. This confirms that the visible wavelengths inducing *L. monocytogenes* inactivation were within the visible wavelength region ranging from 400 to 450 nm.

Bandpass filters (10 nm bandwidth) were subsequently used to identify the narrow bandwidth of visible light between 400 and 450 nm to which the bacteria were most sensitive. Samples exposed to each 10 nm bandwidth between 400 and 450 nm received a dose of 123.3 J cm^{-2}. Data presented in Table 1 demonstrate that statistically significant $\log_{10}$ reductions in bacterial population were achieved through exposure to 400–440 nm bandwidths; however, the bacteria were found to be most sensitive to wavelengths in the region of 400–410 nm, with the highest sensitivity shown with exposure to 405(±5) nm light.

Table 1. Wavelength sensitivity of *Listeria monocytogenes* following exposure to 10 nm bandwidths of light from 400 to 450 nm, each exposed to a dose of 123.3 J cm^{-2}.

Bandwidth (nm)	Irradiance (mW cm^{-2})	Log_{10} (N/N_0) reduction	Statistical significance (P-value ≤0.05)
400 ± 5	7.5	1.05* (±0.00)	0.000
405 ± 5	8.6	1.45* (±0.14)	0.000
410 ± 5	7.2	1.23* (±0.77)	0.001
415 ± 5	7.5	0.51* (±0.63)	0.003
420 ± 5	7.7	0.25* (±0.06)	0.005
430 ± 5	7.3	0.19* (±0.22)	0.031
440 ± 5	8.3	0.11* (±0.06)	0.034
450 ± 5	8.3	0.04 (±0.02)	0.410

*Significant bacterial $\log_{10}$ reductions when compared with the nonexposed control samples, calculated at a 95% confidence interval.

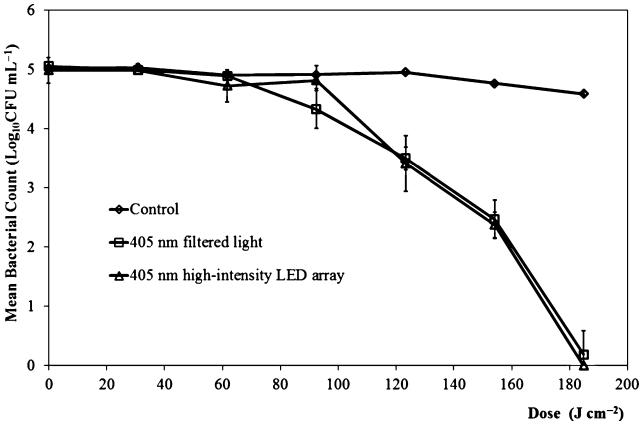

Figure 4. Comparative data for inactivation of *Listeria monocytogenes* through exposure to 405 nm filtered light (using xenon broadband light with a 405[±5] nm bandpass filter), and a 405(±5) nm high-intensity LED array, both with an irradiance of 8.6 mW cm^{-2}. No significant differences were observed between the inactivation kinetics for the light sources.

Comparison of inactivation using 405 nm filtered light and a 405 nm LED array

As a comparison, suspensions of *L. monocytogenes* were exposed to equivalent doses of 405 nm light, using an irradiance level of 8.6 mW cm^{-2} from both the broadband xenon lamp in combination with the 405 nm optical filter (10 nm FWHM), and the 405 nm LED array (14 nm FWHM). The resultant bacterial inactivation data (Fig. 4) show that the same pattern of reduction in bacterial population is shown with exposure to both light sources, with no detectable survival (<1 CFU mL^{-1}) of a 10^5 CFU mL^{-1} population of *L. monocytogenes* observed after exposure to 405 nm light at a dose of 185 J cm^{-2} in both cases. Statistical analysis of the data also determined that there was no significant difference between each of the data points for inactivation using the 405 nm filtered light *versus* the 405-nm LED array.

Effect of an oxygen scavenger on the inactivation process

L. monocytogenes was exposed to 185 J cm^{-2} 405 nm light in the presence of DMTU. Results demonstrated that there was no significant change in the bacterial population when exposed to 405 nm light in the presence of the scavenger DMTU (5.36 $\log_{10}$ CFU mL^{-1} [±0.02 SD] *versus* 5.38 $\log_{10}$ CFU mL^{-1} [±0.02 SD] nonexposed control population). As determined in the previous experiment, exposure to a dose of 185 J cm^{-2} resulted in no detectable survival when bacteria were exposed in the absence of the scavenger.

Dose-dependence experiments

Figure 5 shows the results of dose-dependence experiments for the inactivation of *L. monocytogenes* in liquid suspension through exposure to the 405 nm LED array. It can be observed from Fig. 5 that over the range of applied dose, of approximately 62–154 J cm^{-2}, the inactivation rate of *L. monocytogenes* is relatively similar when the same dose is applied using the four different light intensities. Statistical analysis showed no significant differences in inactivation rates when irradiances of 44.7, 66.1 and 85.6 mWcm^{-2} are compared for the same applied dose. Although inactivation kinetics were similar, statistically significant differences in

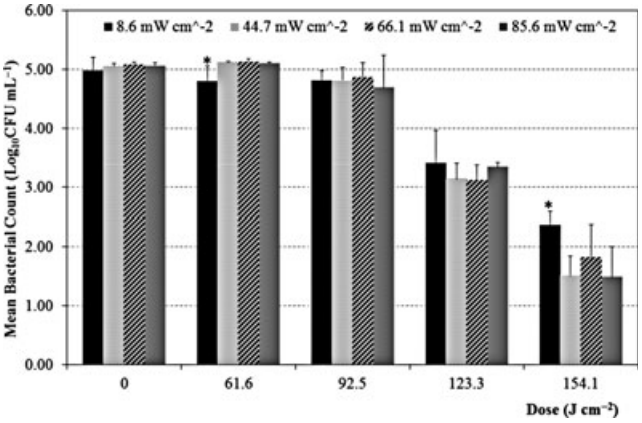

Figure 5. Dose-dependence experiments investigating the inactivation rates of *Listeria monocytogenes* following exposure to a 405 nm high-intensity LED array with differing irradiance levels. * indicates where a light-exposed bacterial count was significantly different from the counts observed using the other irradiances at that particular applied dose ($P \leq 0.05$ calculated at the 95% confidence interval).

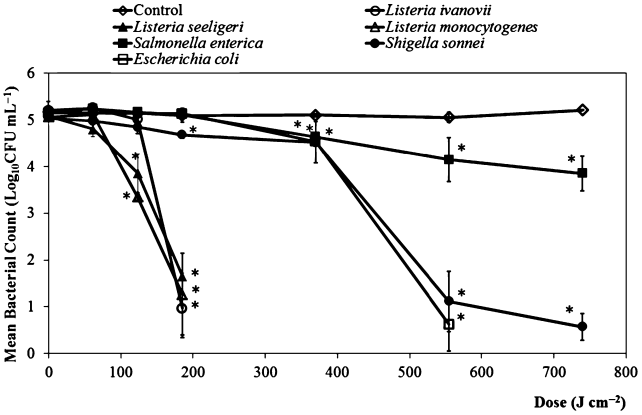

Figure 6. Inactivation of *Listeria* species and other significant foodborne bacterial pathogens by exposure to 405 nm high-intensity light from an LED array (14 nm FWHM) with an irradiance of 85.6 mW cm^{-2}. * indicates where a light-exposed bacterial count was significantly different from the nonexposed control count ($P \leq 0.05$ calculated at the 95% confidence interval).

inactivation rate were, however, found when using an irradiance of 8.6 mWcm^{-2} for bacterial inactivation at doses of 61.6 and 154.1 J cm^{-2}. Overall, however, the results show that, over the range tested, the dose required for bacterial inactivation—regardless of how it is applied—yields similar final populations.

Inactivation of bacteria using a high-intensity 405 nm LED array

Figure 6 shows the results for the inactivation of *L. monocytogenes* through exposure to high-intensity 405 nm light (14 nm FWHM) from an LED array, with an irradiance of 85.6 mW cm^{-2}. For comparative purposes, inactivation data were obtained for two further species of *Listeria* (*L. ivanovii* and *L. seeligeri*) and also for the significant Gram-negative foodborne pathogens *S. enterica*, *Sh. sonnei* and *E. coli* 0157:H7. From Fig. 6, it can be seen that exposure to a dose of 184.9 J cm^{-2} results in similar inactivation kinetics for all three *Listeria* species tested, with 3.72 and 3.31 $\log_{10}$ reductions of *L. monocytogenes* and *L. seeligeri*, respectively, and near-complete inactivation of *L. ivanovii*, which demonstrated a 4.12 $\log_{10}$ reduction in bacterial population. Exposure of suspensions of *E. coli* and *Sh. sonnei* to a dose of 554.7 J cm^{-2} resulted in 4.52 $\log_{10}$ and 3.9 $\log_{10}$ reductions, respectively. Inactivation of *S. enterica*, however, required exposure to higher doses of 405 nm light, with reductions of 1.36 $\log_{10}$ after a total dose of 739.6 J cm^{-2}. The control trend line in Fig. 6 is an average of the data from all experiments and demonstrates that the unexposed populations show no significant change over the duration of the experiments.

In addition to this, PBS exposed to 739.6 J cm^{-2} (the maximum dose of light exposure used in this study) did not become bactericidal, with no change in population evident between bacteria added to the exposed PBS, when compared with the nonexposed PBS control, thus demonstrating that the bactericidal effect was not caused by any light-induced changes to the PBS-suspending medium.

DISCUSSION

The combination of optical filters used in this study demonstrated that the most effective visible-light wavelengths for the inactivation of *L. monocytogenes* were in the violet/blue region between 400 and 450 nm. Comparison of inactivation results using light comprised of all wavelengths >400 nm emitted by the broadband source with results obtained using light restricted to the 400–500 nm range also demonstrated that the violet/blue light inactivation was not enhanced by the presence of the longer wavelengths. When the transmitted light was restricted to wavelengths >450 nm, no inactivation was observed. Subsequent analysis of wavelengths between 400 and 450 nm demonstrated that *L. monocytogenes* was most sensitive to wavelengths of between 400 (±5) and 410 (±5) nm, with highest sensitivity using 405 (±5) nm wavelength light. Similar inactivation results were obtained using two different sources of 405 nm light, one being from a 405 nm LED array (14 nm FWHM) and the other 405 nm filtered light (10 nm FWHM) from a broadband xenon lamp, demonstrating that the applied dose of 405 nm light is the important factor, not other properties associated with the different light sources. A previous visible-light wavelength sensitivity study of another Gram-positive bacterium, *Staphylococcus aureus*, by Maclean *et al.* (13) also found 405(±5) nm light to be an effective wavelength for inactivation without the use of exogenous photosensitizers.

Exposure of other *Listeria* species and the foodborne pathogens *E. coli*, *S. enterica* and *Sh. sonnei* to 405 nm light also demonstrated significant bacterial inactivation, with the inactivation kinetics of all the organisms investigated in this study showing a similar sigmoidal shape to those from previous studies on the bactericidal effect of 405 nm light (12,13,18). Treatment of *L. monocytogenes* and the other *Listeria* species using the 405 nm LED array resulted in similar inactivation kinetics, suggesting that bacteria within the same genus may undergo very similar inactivation reactions. When compared with the Gram-negative foodborne pathogens, *Listeria* had higher susceptibility to inactivation through 405 nm light exposure and a 5 $\log_{10}$ reduction was achieved

upon application of a dose of 185 Jcm^{-2}. Suspensions of *E. coli* and *Sh. sonnei* required three to four times the dose for log reductions similar to those achieved with *Listeria*. *S. enterica* was found to be the least susceptible of the organisms tested, with a reduction of approximately 1.3 $\log_{10}$ after exposure to four times the dose (740 J cm^{-2}). This is similar to the trend found in the study by Maclean *et al.* (12), which showed that, in general, Gram-positive organisms required lower doses of 405 nm light exposure for inactivation. A previous study proposed that these different inactivation rates may be the result of bacterial species producing differing types and levels of intracellular porphyrins that can act as photosensitizer molecules (19). If the peak absorption wavelengths of these porphyrins differ, then different wavelengths may be required for their optimum photostimulation.

In order to determine that the inactivation of *Listeria* and the other organisms in this study using 405 nm light was a result of an endogenous photodynamic reaction, it was important to demonstrate that light exposure of PBS, the bacterial suspending medium, did not induce the observed bactericidal effects. Results demonstrated no bactericidal effects of light-exposed PBS. In the case of photosensitization studies that involve tissue treatment using photosensitizer dyes, it would be difficult to distinguish between what was happening exogenously and endogenously. However in this study, bacteria were exposed while suspended in PBS, and as no photochemical biocidal effect was produced in the PBS, the photoinactivation observed in this study must be endogenous, *i.e.* caused by chemicals associated with the bacteria.

There is limited existing data in the literature investigating the inactivation of *Listeria* through visible-light exposure, either with or without the addition of exogenous photosensitizers, and as far as the authors are aware, this study presents for the first time the inactivation of *Listeria* species through exposure to 405 nm light without the addition of photosensitizer molecules or pretreatments. Several published studies have suggested the role of endogenous porphyrin molecules as the photosensitizer facilitating bacterial inactivation using blue/visible light (12,14,20,21), and consequently a limitation of the present work was that the content of endogenous porphyrins within the bacteria was unable to be measured; however, porphyrins are present in most microorganisms including *L. monocytogenes*, which is known to possess porphyrin hemoproteins including cytochromes and catalase (22,23). Although the metal component of hemoproteins quenches ROS and prevents participation in photosensitizing reactions, their presence demonstrates that *Listeria* possesses the porphyrin biosynthetic pathway and this is further evidenced as *Listeria* produce endogenous photosensitizers in the presence of exogenously supplied 5-aminolevulinic acid (ALA) (24,25) with ALA being a key precursor in the biosynthesis of microbial porphyrins (26). Further evidence supporting the inactivation mechanism observed in the current study being photodynamic inactivation through the excitation of endogenous molecules is the nonreaction observed when the bacteria were exposed in the presence of a scavenger. Photodynamic inactivation requires oxygen, and results in the generation of a variety of ROS toxic to the bacterial cells (27). The demonstration that inactivation did not occur in the presence of the scavenger demonstrates that ROS are essential for this process, and supports results documented in other published papers investigating the visible-light inactivation of bacterial species (28,29).

The sensitivity of *L. monocytogenes* to 405($\pm$5) nm light can also be seen by comparing the relative biocidal effectiveness of the transmitted light ranges in Figures 3 and 4. When a dose of 185 J cm^{-2} is delivered through a narrow bandpass filter or a 405 nm LED array (ensuring that the entire dose is comprised of 405[$\pm$5] nm light), a 5 $\log_{10}$ inactivation was achieved. However, when similar or indeed much higher light doses are delivered through various LWP and SWP filters, inactivation is much poorer and this simply reflects the much smaller amount of bactericidal photons with the wavelength 405($\pm$5) nm and the much greater amount of nonbiocidal photons that are present in the light dose delivered through these LWP and SWP filters. In the case of the LWP and SWP filters, the spectral irradiance, E in Eq. (1), is a measurement over the whole broadband wavelength range transmitted through these filters (*e.g.* >400, 400–500, >450, <500 nm). Conversely, the 405 nm narrow bandpass filter only allows the transmission of a 10 nm bandwidth, therefore the value of irradiance, E, is obtained over this 10 nm bandwidth. Therefore, for the same dose of 185 J cm^{-2}, the number of photons with a wavelength of 405 nm will be significantly higher in the case of the narrow bandpass filter than in the case of the broadband filters. This explains the observed difference in the inactivation efficiency of the same light dose of 185 Jcm^{-2} with the different spectral content.

Previous work on the visible-light inactivation of *Listeria* has involved preincubation with molecules that either increase the synthesis of endogenous porphyrin levels prior to light exposure or act as photosensitizing agents themselves. Vaitonis and Lukšienė (25) used high-powered LEDs with peak wavelength at 400 nm and preincubation with ALA to achieve photosensitization inactivation of several pathogens including *Listeria* on food surfaces. Also Buchovec *et al.* (24) reported that 7.5 mM ALA used in conjunction with a 400 nm LED-based light source could be used to inactivate *L. monocytogenes*. They found a 3.7 $\log_{10}$ reduction on packaging material, 3.1 $\log_{10}$ reduction in bacterial biofilm and 4 $\log_{10}$ reduction in suspension after exposure to a dose of 20 J cm^{-2}. Similar approaches using preincubation with ALA to achieve photosensitization-based inactivation has also been used to achieve enhanced inactivation of other bacteria including *Bacillus cereus* (30) and *S. enterica* (31).

An alternative photodynamic treatment approach is the use of exogenously added photoactive substances. Studies by Brovko *et al.* and Brovko (32,33) have described a photodynamic method for the cleaning and disinfection of surfaces that utilizes various nontoxic dyes as the photosensitizer. Protochlorophyllide, the natural intermediate of chlorophyll biosynthesis, has also been used as a photosensitizer for the inactivation of various microorganisms including *L. monocytogenes* (34). In another study (35), a 405 nm LED, with an intensity of 20 mW cm^{-2}, was used in conjunction with Na-Chlorophyllin (Na-Chl)-based photosensitization for the inactivation of *L. monocytogenes* in PBS suspension and on packaging surfaces. They found that with a dose of 36 J cm^{-2}, *Listeria* in suspension could be inactivated by 7 $\log_{10}$, and for packaging surfaces, results demonstrated that Na-Chl-based photosensitization was much more effective against *Listeria* than washing with water or 200 ppm sodium-hypochlorite,

with respective $\log_{10}$ reductions in bacterial population of 4.5, 1.7 and < 1 being achieved (35). A 400 nm LED-based light source along with Na-Chl-based photosensitization have also been used for decontamination of the surface of strawberries, with seeded populations of *L. monocytogenes* reduced by up to 98% after 30 min exposure to an irradiance of 20 mW cm^{-2} (36).

The results of the aforementioned studies highlight the accelerated inactivation rates that can be achieved when exogenous photosensitizer molecules are incorporated into the system. The present work has highlighted the bactericidal sensitivity of *Listeria* species to 405 nm light and, compared to exposures involving exogenous photosensitizing agents, relatively high doses are required for inactivation. However, the fact that this inactivation can be achieved solely through exposure to 405 nm light without relying on the addition of exogenous chemicals makes this approach a potentially interesting alternative for certain practical applications. The demonstration that the inactivation process is dose-dependent also means that the inactivation of bacteria can be achieved using both low-intensity light applied over longer exposure times and high-intensity light over more rapid exposure times, giving it flexibility in how it can be applied. This study was limited by the light output of the LED array used; however, development of systems that use multiple- or high-power LED arrays/light sources could enable the delivery of very high doses of 405 nm light for localized decontamination applications.

It is also significant that 405 nm light, which falls within the visible-light region, does not require the same level of safety exposure precautions associated with the use of UV light. The application of UV light for inactivation of the foodborne pathogen *Listeria* has been studied widely (37–42). Rapid inactivation rates are achievable through the use of UV light; however, the comparative safety advantage of 405 nm light over UV light for some applications has now been demonstrated by studies in the clinical environment (43,44), where it has been shown that 405 nm light can be used to achieve enhanced decontamination of exposed surfaces in isolation rooms over continuous periods in the presence of both patients and staff. Lighting technology that utilizes the bactericidal properties of 405 nm light may also have potential applications in other areas including the food industry sector, and the results of this study, which demonstrate the sensitivity of the significant foodborne pathogen *L. monocytogene*s, support this potential application.

Acknowledgements—The first author would like to thank the Indonesian Government, the Ministry of National Education, Directorate General of Higher Education (DIKTI) and Physics Department, Institut Teknologi Sepuluh Nopember (ITS) Surabaya, Indonesia, for providing the scholarship. All authors would like to thank The Robertson Trust for their funding support.

REFERENCES

1. Malik, Z., J. Hanania and Y. Nitzan (1990) New trends in photobiology bactericidal effects of photoactivated porphyrins—An alternative approach to antimicrobial drugs. *J. Photochem. Photobiol. B: Biol.* **5**, 281–293.
2. O'Neill, J. F., C. K. Hope and M. Wilson (2002) Oral bacteria in multi-species biofilms can be killed by red light in the presence of toluidine blue. *Lasers Surg. Med.* **31**, 86–90.
3. Soukos, N. S., L. A. Ximenez-Fyvie, M. R. Hamblin, S. S. Socransky and T. Hasan (1998) Targeted antimicrobial photochemotherapy. *Antimicrob. Agents Chemother.* **42**, 2595–2601.
4. Papageorgiou, P., A. Katasambas and A. Chu (2000) Phototherapy with blue (415 nm) and red (660 nm) light in the treatment of acne vulgaris. *Br. J. Dermatol.* **142**, 973–978.
5. Lipovsky, A., Y. Nitzan, H. Friedmann and R. Lubart (2009) Sensitivity of *Staphylococcus aureus* strains to broadband visible light. *J. Photochem. Photobiol.* **85**, 255–260.
6. Wainwright, M. (1998) Photodynamic antimicrobial chemotherapy (PACT). *J. Antimicrob. Chemother.* **42**, 13–28.
7. Zeina, B., J. Greenman, W. M. Purcell and B. Das (2001) Killing of cutaneous microbial species by photodynamic therapy. *Br. J. Dermatol.* **144**, 274–278.
8. Friedberg, J. S., C. Skema, E. D. Baum, J. Burdick, S. A. Vinogradov, D. F. Wilson, A. D. Horan and I. Nachamkin (2001) *In vitro* effects on photodynamic therapy on *Aspergillus fumigatus*. *J. Antimicrob. Chemother.* **48**, 105–107.
9. Lambrechts, S. A. G., M. C. G. Aalders and J. Van Marle (2005) Mechanistic study of the photodynamic inactivation of *Candida albicans* by a cationic porphyrin. *Antimicrob. Agents Chemother.* **49**, 2026–2034.
10. Bachmann, B., J. Knüver-Hopf, B. Lambrecht and H. Mohr (1995) Target structures for HIV-1 inactivation by methylene blue and light. *J. Med. Virol.* **47**, 172–178.
11. Ferro, S., O. Coppellotti, G. Roncucci, T. Ben Amor and G. Jori (2006) Photosensitized inactivation of *Acanthamoeba palestinensis* in the cystic stage. *J. Appl. Microbiol.* **101**, 206–212.
12. Maclean, M., S. J. MacGregor, J. G. Anderson and G. Woolsey (2009) Inactivation of bacterial pathogens following exposure to light from a 405-nanometer Light-Emitting Diode array. *Appl. Environ. Microbiol.* **75**, 1932–1937.
13. Maclean, M., S. J. MacGregor, J. G. Anderson and G. Woolsey (2008) High-intensity narrow-spectrum light inactivation and wavelength sensitivity of *Staphylococcus aureus*. *FEMS Microbiol. Lett.* **285**, 227–232.
14. Elman, M., M. Slatkine and Y. Harth (2003) The effective treatment of acne vulgaris by a high-intensity, narrow band 405–420 nm light source. *J. Cosmet. Laser Ther.* **5**, 111–117.
15. FDA (2003) Quantitative assessment of relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of Ready-to-Eat foods. Available at: http://www.fda.gov/downloads/food/scienceresearch/researchareas/riskassessmentsafetyassessment/ucm197330.pdf. Accessed on 10 March 2011.
16. Mead, P. S., L. Slutsker, V. Dietz, L. F. McCaig, J. S. Bresee, C. Shapiro, P. M. Griffin and R. V. Tauxe (1999) Food-related illness and death in the United States. *Emerg. Infect. Dis.* **5**, 607–625.
17. Sliney, D., D. Aron-Rosa, F. DeLori, F. Fankhauser, R. Landry, M. Mainster, J. Marshall, B. Rassow, B. Stuck, S. Trokel, T. M. West and M. Wolffe (2005) Adjustment of guidelines for exposure of the eye to optical radiation from ocular instruments: Statement from a task group of the International Commission on Non-Ionizing Radiation Protection (ICNIRP). *Appl. Opt.* **44**, 2162–2176.
18. Murdoch, L. E., M. Maclean, S. J. MacGregor and J. G. Anderson (2010) Inactivation of *Campylobacter jejuni* by exposure to high-intensity 405-nm visible light. *Foodborne Pathog Dis.* **7**, 1211–1216.
19. Nitzan, Y., M. Salmon-Divon and E. Shporen, Z. Malik (2004) ALA induced photodynamic effects on Gram positive and negative bacteria. *Photochem. Photobiol. Sci.*. **3** (5), 430–435.
20. Ganz, R. A., J. Viveiros, A. Ahmad, A. Ahmadi, A. Khalil, M. J. Tolkoff, N. S. Nishioka and M. R. Hamblin (2005) *Helicobacter pylori* in patients can be killed by visible light. *Lasers Surg. Med.* **36**, 260–265.
21. Ashkenazi, H., Z. Malik, Y. Harth and Y. Nitzan (2003) Eradication of *Propionibacterium acnes* by its endogenic porphyrins after illumination with high intensity blue light. *FEMS Immunol. Med. Microbiol.* **35**, 17–24.
22. Feresu, S. B. and D. Jones (1988) Taxonomic studies on *Brochothrix, Erysipelothrix, Listeria* and atypical lactobacilli. *J. Gen. Microbiol.* **134**, 1165–1183.

23. Holt, J. G., N. R. Krieg, P. H. A. Sneath, J. T. Staley and S. T. Williams (1994) *Bergey's Manual of Determinative Bacteriology*. Lippincott Williams & Wilkins, Balitmore, CA.
24. Buchovec, I., E. Paskeviciute and Z. Luksiene (2010) Photosensitization-based inactivation of food pathogen *Listeria monocytogenes in vitro* and on the surface of packaging material. *J. Photochem. Photobiol. B: Biol.* **99**, 9–14.
25. Vaitonis, Z. and Z. Lukšienė (2010) LED-based light sources for decontamination of food: Modelling photosensitization-based inactivation of pathogenic bacteria. *Lithuanian J. Physics* **50**, 141–145.
26. Rose, A. H. (1965) *Chemical Microbiology*. Butterworth & Co. (Publishers) Ltd., London, UK.
27. Hamblin, M. R. and T. Hasan (2004) Photodynamic therapy: A new antimicrobial approach to infectious disease? *Photochem. Photobiol. Sci.* **3**, 436–450.
28. Feuerstein, O., I. Ginsburg, E. Dayan, D. Veler and E. I. Weiss (2005) Mechanism of visible light phototoxicity on *Porphyromonas gingivalis* and *Fusobacterium nucleatum*. *Photochem. Photobiol.* **81** (5), 1186–1189.
29. Maclean, M., S. J. MacGregor, J. G. Anderson and G. A. Woolsey (2008) The role of oxygen in the visible-light inactivation of *Staphylococcus aureus*. *J. Photochem. Photobiol., B* **90**, 180–184.
30. Le Marc, Y., I. Buchovec, S. M. George, J. Baranyi and Z. Luksiene (2009) Modelling the photosensitization-based inactivation of *Bacillus cereus*. *J. Appl. Microbiol.* **107**, 1006–1011.
31. Buchevoc, L., Z. Vaitonis and Z. Luksiene (2009) Novel approach to control *Salmonella enterica* by modern biophotonic technology: Photosensitization. *J. Appl. Microbiol.* **106** (3), 748–754.
32. Brovko, L. Y., A. Meyer, A. S. Tiwana, W. Chen, H. Liu, C. D. Filipe and M. W. Griffiths (2009) Photodynamic treatment: A novel method for sanitation of food handling and food processing surfaces. *J. Food Prot.* **72** (5), 1020–1024.
33. Brovko, L. (2010) Photodynamic treatment a new efficient alternative for surface sanitation. *Adv. Food Nutr. Res.* **61**, 119–147.
34. Walther, J., M. J. Bröcker, D. Wätzlich, M. Nimtz, M. Rohde, D. Jahn and J. Moser (2009) Protochlorophyllide: A new photosensitizer for the photodynamic inactivation of Gram-positive and Gram-negative bacteria. *FEMS Microbiol. Lett.* **290** (2), 156–163.
35. Luksiene, Z., I. Buchovec and E. Paskeviciute (2010) Inactivation of several strains of *Listeria monocytogenes* attached to the surface of packaging material by Na-Chlorophyllin-based photosensitization. *J. Photochem. Photobiol. B: Biol.* **101**, 326–331.
36. Paskeviciute, E. and Z. Luksiene (2009) Effective non-thermal photosensitization-based decontamination of strawberries from microorganisms. *Sodininkystėir Daržininkystė* **28**, 89–97.
37. Chun, H., J. Kim, K. Chung, M. Won and K. B. Song (2009) Inactivation kinetics of *Listeria monocytogenes*, *Salmonella enterica serovar Typhimurium*, and *Campylobacter jejuni* in ready-to-eat sliced ham using UV-C irradiation. *Meat Sci.* **83**, 599–603.
38. Sommers, C. H., P. H. Cooke, X. Fan and J. E. Sites (2009) Ultraviolet light (254 nm) inactivation of *Listeria monocytogenes* on frankfurters that contain potassium lactate and sodium diacetate. *J. Food Sci.* **74**, M114–M119.
39. Keklik, N. M., A. Demirci and V. M. Puri (2009) Inactivation of *Listeria monocytogenes* on unpackaged and vacuum-packaged chicken frankfurters using pulsed UV-light. *J. Food Sci.* **74**, M431–M439.
40. Yousef, A. H. E. and E. L. H. Marth (1988) Inactivation of *Listeria monocytogenes* by ultraviolet energy. *J. Food Sci.* **53**, 571–573.
41. McKinney, J., R. C. Williams, G. D. Boardman, J. D. Eifert and S. Sumner (2009) Dose of UV light required to inactivate *Listeria monocytogenes* in distilled water, fresh brine, and spent brine. *J. Food Prot.* **72**, 2144–2150.
42. Gailunas, K. M., K. E. Matak, R. R. Boyer, C. Z. Alvarado, R. C. Williams and S. S. Sumner (2008) Use of UV light for the inactivation of *Listeria monocytogenes* and lactic acid bacteria species in recirculated chill brines. *J. Food Prot.* **71**, 629–633.
43. Maclean, M., S. J. MacGregor, J. G. Anderson, G. A. Woolsey, J. E. Coia, K. Hamilton, I. Taggart, S. B. Watson, B. Thakker and G. Gettinby (2010) Environmental decontamination of a hospital isolation room using high-intensity narrow-spectrum light. *J. Hosp. Infect.* **76** (3), 247–251.
44. Bache, S. E., M. Maclean, S. J. MacGregor, J. G. Anderson, G. Gettinby, J. E. Coia and I. Taggart (2012) Clinical studies of the High-Intensity Narrow-Spectrum light Environmental Decontamination System (HINS-light EDS) for continuous disinfection in the burn unit inpatient and outpatient settings. *Burns* **38**, 69–76.