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Inactivation of *Campylobacter jejuni* by Exposure to High-Intensity 405-nm Visible Light

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Abstract

Although considerable research has been carried out on a range of environmental factors that impact on the survival of *Campylobacter jejuni*, there is limited information on the effects of violet/blue light on this pathogen. This investigation was carried out to determine the effects of high-intensity 405-nm light on *C. jejuni* and to compare this with the effects on two other important Gram-negative enteric pathogens, *Salmonella enteritidis* and *Escherichia coli* O157:H7. High-intensity 405-nm light generated from an array of 405-nm light-emitting diodes was used to inactivate the test bacteria. The results demonstrated that while all three tested species were susceptible to 405-nm light inactivation, *C. jejuni* was by far the most sensitive organism, requiring a total dose of 18 J cm^{-2} of 405-nm light to achieve a 5-log₁₀ reduction. This study has established that *C. jejuni* is particularly susceptible to violet/blue light at a wavelength of 405 nm. This finding, coupled with the safety-in-use advantages of this visible (non-ultraviolet wavelength) light, suggests that high-intensity 405-nm light may have applications for control of *C. jejuni* contamination levels in situations where this type of illumination can be effectively applied.

Introduction

DESPITE INTENSIVE RESEARCH EFFORT, *Campylobacter jejuni* remains the most significant cause of bacterial gastroenteritis in the United Kingdom and United States with around 45,000 and 2–4 million cases of *Campylobacter*-related foodborne illness per annum, respectively (ACMSF, 2005; FDA, 2009). *C. jejuni* is also a major cause of food-related morbidity worldwide (WHO, 2009). Infection by *C. jejuni* is important not only because it is a cause of acute gastroenteritis but also because of the associated risk of postsequelae illness such as Guillain–Barre and Miller Fisher syndromes (Rhodes and Tatterfield, 1982; Servan *et al.*, 1995).

In comparison with other enteric bacterial pathogens, *C. jejuni* has a number of unusual features that affect its survival in the *in vitro* environment. The importance of temperature on growth and survival is well documented (El-Shibiny *et al.*, 2009; Hughes *et al.*, 2009) as is information on factors such as oxygen-related toxicity (Hoffman *et al.*, 1979; Bolton *et al.*, 1984; Juven and Rosenthal, 1985; Verhoeff-Bakkenes *et al.*, 2008; Attack and Kelly, 2009), acidity (Reid *et al.*, 2008), osmolarity (Reezal *et al.*, 1998), and nutritional availability (Klancnik *et al.*, 2009), among others.

Light is another potentially inactivating factor that can affect the survival of enteric pathogens in natural environments. Previous research has been carried on the effects of

ultraviolet-B (UV-B) light (280–315 nm) from natural and artificial sources on *C. jejuni* with regard to survival of the organism in river and coastal waters and in sewage effluent (Jones *et al.*, 1990; Wallace *et al.*, 1994; Obiri-Danso *et al.*, 2001). In a study of the susceptibility of *C. jejuni* to UV-C radiation at 254 nm, it was reported that *C. jejuni* was more sensitive to UV than many other pathogens (Butler *et al.*, 1987). There has also been specific interest in the use of UV-C irradiation for the reduction of *C. jejuni* on broiler meat (Isohanni and Lyhs, 2009), chicken breasts (Chun *et al.*, 2010), and ready-to-eat sliced ham (Chun *et al.*, 2009). While UV light effectively inactivates microorganisms on exposed surfaces, it suffers from poor penetrability into solid materials and opaque liquids. UV light can also have harmful effects on the skin and eyes of the operator, thereby further limiting its use as a decontamination technology in the food-processing and preparation environments.

Studies have shown that bacteria can be photodynamically inactivated solely through exposure to visible light, specifically blue light, by an oxygen-dependent process accredited to the photostimulation of endogenous porphyrin molecules, which causes energy transfer and production of reactive oxygen species (ROS) that are bactericidal (Maclean *et al.*, 2008b). *Propionibacterium acnes*, *Helicobacter pylori*, oral black-pigmented bacteria, and other bacteria with high levels of natural endogenous porphyrins have been investigated

with regard to blue light sensitivity (Ashkenazi *et al.*, 2003; Elman and Lebzeltzer, 2004; Hamblin *et al.*, 2005; Soukos *et al.*, 2005). More recent studies using 405-nm blue light have shown inactivation of a range of medically important bacteria from the genera *Staphylococcus*, *Clostridium*, *Acinetobacter*, and *Pseudomonas* (Guffey and Wilborn, 2006; Maclean, 2006; Enwemeka *et al.*, 2008; Maclean *et al.*, 2008a, 2009).

The present study investigates the effect of high-intensity 405-nm light exposure on *C. jejuni* in the absence of exogenous photosensitizing molecules. In addition, the sensitivity of *C. jejuni* to 405-nm light was compared with the sensitivities of *Salmonella enteritidis* and *Escherichia coli* O157:H7, two of the most prevalent causative microorganisms associated with diarrhoeal disease in both the United Kingdom and the United States (FDA, 2009; FSA, 2009). The use of these bacteria, which are also Gram-negative food-related pathogens, allowed comparison of the inactivation kinetics of *Campylobacter*, an oxygen-sensitive microaerophilic organism, with those of oxygen-tolerant facultative anaerobic enteric pathogens.

Materials and Methods

Microorganisms

C. jejuni LMG 8841 was obtained from The Belgian Co-ordinated Collections of Microorganisms, Gent, Belgium. *S. enteritidis* NCTC 4444 and *E. coli* serotype O157:H7 NCTC 12900 were obtained from the National Collection of Type Cultures, Collindale, United Kingdom. *C. jejuni* was cultured on blood agar plates and incubated at 42°C in gas jars with CampyGen™ gas-producing sachets (Oxoid). After 48 hours, the surface growth was suspended in phosphate-buffered saline (PBS) and diluted to give a starting population of $\sim 10^5$ colony forming units per milliliter (CFU mL⁻¹). *S. enteritidis* and *E. coli* O157:H7 were inoculated into 100 mL of nutrient broth (Oxoid). Broths were cultivated at 37°C for 18 hours in a rotary incubator (120 rpm) and then centrifuged at 3939g for 10 minutes. The pellet was resuspended in 100 mL of PBS and diluted to give a starting population of $\sim 10^5$ CFU mL⁻¹.

405-nm light-emitting diode light source

High-intensity 405-nm light was produced by an indium-gallium-nitride (InGaN) 99-DIE light-emitting diode (LED)

array (Opto Diode Corp.), with a center wavelength of around 405 nm and a bandwidth of ~ 10 nm at full half width maximum. A cooling fan and heat sink were attached to the array to dissipate heat from the source, which also served to minimize heat transfer to the sample such that the sample temperature remained in the range of 25°C–27°C throughout treatment. As shown in Figure 1, the LED array was mounted in a polyvinyl chloride (PVC) housing designed to fit a 12-well micro-plate (Nunc), with the array positioned directly above one of the central sample wells. The LED array was powered by a DC power supply (0–3 A and 0–15 V). Current was set at 0.5 A (± 0.05) at a voltage of 11.2 V (± 0.2), giving an approximate irradiance of 10 mW cm⁻² at the surface of the bacterial suspension.

Exposure experiments

A 2-mL volume of bacterial suspension was pipetted into a micro-plate well giving a liquid depth of ~ 7 mm. A micro-magnetic follower (7 × 2 mm) was placed in the well, and the plate placed onto a magnetic stirring plate for continuous agitation of the sample. The LED array (in housing) was set at the required irradiance of 10 mW cm⁻², and placed directly over the open (without lid) sample well. A second multi-dish was set up exactly as described but received no high-intensity 405-nm light and served as a control. Test samples were exposed to increasing durations of high-intensity 405-nm light, and control samples were obtained for each time exposure investigated. At the population densities ($\sim 10^5$ CFU mL⁻¹) and sample depths (7 mm) used in this study, there was no measurable attenuation of light irradiance through the bacterial suspension, and therefore the 10 mW cm⁻² irradiance can be considered to be constant throughout the entire sample depth (Maclean *et al.*, 2009). Irradiance was measured using a radiant power meter (L.O.T.-Oriol Ltd.) in conjunction with a photodiode detector. The dose of 405-nm light that each bacterial sample was exposed to was calculated using Equation 1:

$$E' = P't \quad (1)$$

where E' = energy density (dose) in J cm⁻², P' = power density (irradiance) in W cm⁻², and t = time in seconds.

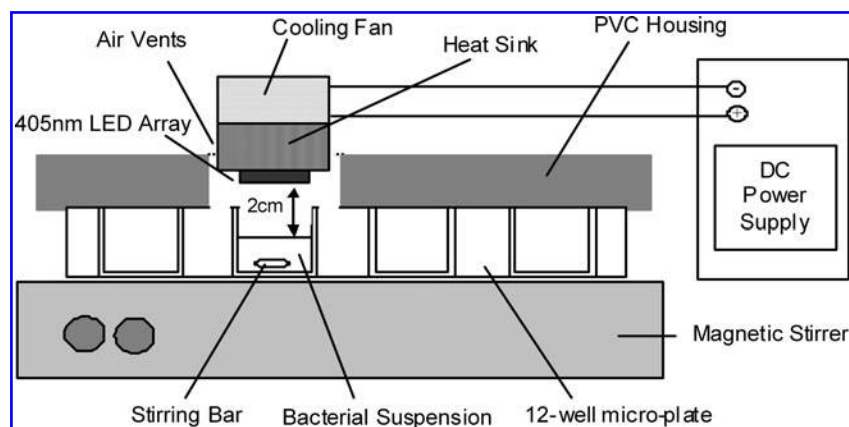


FIG. 1. Diagram of the experimental arrangement during treatment of bacterial suspensions with high-intensity 405-nm light. LED, light emitting diode.

TABLE 1. EFFECT OF HIGH-INTENSITY 405-NM LIGHT EXPOSURE DOSE ON THE INACTIVATION OF *CAMPYLOBACTER JEJUNI*

Exposure time (min)	Dose (J cm ⁻²)	Light-exposed population, N (log ₁₀ CFU mL ⁻¹)	Nonexposed control population, N ₀ (log ₁₀ CFU mL ⁻¹)	Log ₁₀ (N/N ₀) reduction	Statistical significance
0	0	–	5.60 (±0.06)	–	–
5	3	5.49 (±0.03)	5.67 (±0.08)	0.18	0.135
10	6	5.05 (±0.01)	5.59 (±0.03)	0.54	0.004 ^a
15	9	4.55 (±0.13)	5.66 (±0.05)	1.11	0.009 ^a
20	12	3.92 (±0.20)	5.40 (±0.01)	1.48	0.001 ^a
25	15	3.24 (±0.20)	5.50 (±0.005)	2.26	0.00 ^a
30	18	ND (±0.0)	5.25 (±0.03)	5.25	0.003 ^a

^aLight-exposed sample value was significantly different from nonexposed control value ($p \leq 0.05$ calculated at the 95% confidence interval). ND, nondetectable (i.e., bacterial levels were <1 CFU mL⁻¹); CFU mL⁻¹, colony forming units per milliliter.

Plating and enumeration

After exposure, test and control samples were plated onto agar plates. A WASP 2 spiral plater (Don Whately Scientific Ltd.) was used to plate out the samples, either as 50-μL spiral plate or 100-μL spread plate samples, with each sample being plated in triplicate (at least). Pour plates of the samples were prepared if low counts were expected, as this allowed a larger volume (1 mL) of the test sample to be enumerated, thereby providing a detection limit of 1 CFU mL⁻¹.

Test and control samples of *C. jejuni* were plated onto blood agar, placed in a gas jar containing a CampyGen™ sachet and incubated at 42°C for 48 hours. *S. enteritidis* and *E. coli* O157:H7 samples were plated onto nutrient agar and incubated at 37°C for 24 hours. After incubation the plates were enumerated and results reported as CFU mL⁻¹.

Statistical analysis

In the 405-nm light exposure experiments, data points on each figure represent the mean results of two or more independent experiments, with each individual experimental data point being sampled in triplicate at least. Data points also

contain the standard deviation and significant differences attained from results. Significant differences were calculated at the 95% confidence interval using analysis of variance (one-way) with MINITAB software release 15.

Results

Response of test bacteria to 405-nm light dose

Table 1 shows the log₁₀ reduction values obtained when exposing *C. jejuni* to increasing dosages of high-intensity 405-nm light in liquid suspension. Statistically significant differences between the nonexposed control samples and the light-exposed samples were seen after exposure to doses of 6 J cm⁻² and above. Inactivation of *C. jejuni* by 5 log₁₀ CFU mL⁻¹ to nondetectable levels (<1 CFU mL⁻¹) was obtained upon exposure to 405-nm light at a total dose of 18 J cm⁻². Figure 2 shows the collated inactivation curves of *C. jejuni*, *S. enteritidis*, and *E. coli* O157:H7 upon exposure to high-intensity 405-nm light. Results demonstrate that although susceptible to inactivation through 405-nm light exposure, significantly higher doses were required to achieve this inactivation effect. *C. jejuni* was inactivated to nondetectable levels

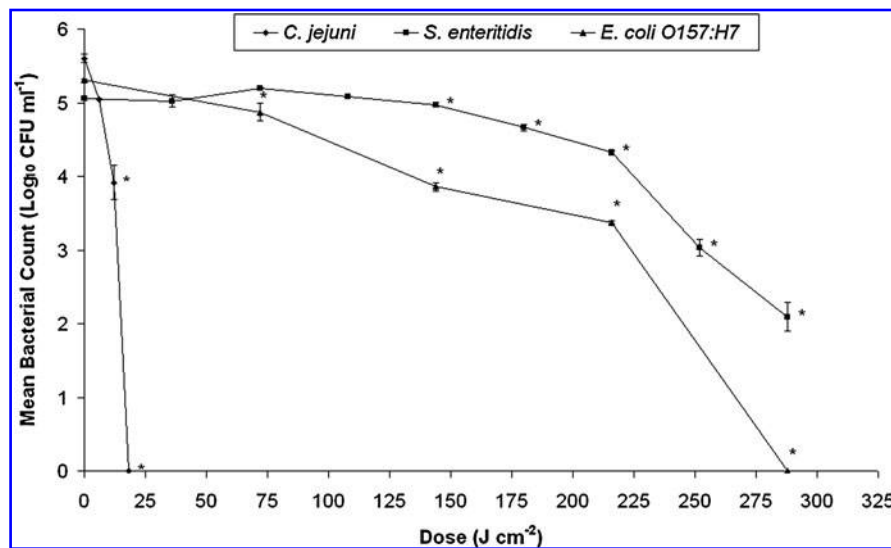


FIG. 2. Comparison of the high-intensity 405-nm light inactivation of *Campylobacter jejuni* with two other Gram-negative bacteria, *Salmonella enteritidis* and *Escherichia coli* O157:H7, at a light irradiance of ~10 mW cm⁻². Asterisk indicates light-exposed samples that were significantly different from control samples ($p \leq 0.05$). Control values remained constant throughout experimentation in all three cases.

TABLE 2. LOG₁₀ REDUCTION AND GERMICIDAL EFFICIENCY VALUES FOR THE INACTIVATION OF A RANGE OF FOODBORNE PATHOGENS THROUGH HIGH-INTENSITY 405-NM LIGHT EXPOSURE

Organism	Initial population N ₀ (log ₁₀ CFU mL ⁻¹)	Final population N (log ₁₀ CFU mL ⁻¹)	Log ₁₀ (N/N ₀) reduction	Dose (J cm ⁻²)	Germicidal efficiency η [log ₁₀ (N/N ₀) J cm ⁻²]
<i>Campylobacter jejuni</i>	5.25 (±0.06)	ND (±0.0)	5.25	18	0.30 (0.0%) ^a
<i>Salmonella enteritidis</i>	5.06 (±0.02)	2.10 (±0.2)	2.96	288	0.01 (±0.01%) ^a
<i>Escherichia coli</i> O157:H7	5.30 (±0.02)	ND (±0.0)	5.30	288	0.02 (±0.0%) ^a

^aPercentage uncertainty of germicidal efficiency values obtained from experimental results calculated to three decimal places. A percentage uncertainty value of 0.0% indicates that there was no difference between germicidal efficiency values.

ND, nondetectable (i.e., bacterial levels were <1 CFU mL⁻¹).

(<1 CFU mL⁻¹) after a total dose of 18 J cm⁻², whereas *S. enteritidis* and *E. coli* O157:H7 were inactivated by around 3 log₁₀ CFU mL⁻¹ and 5 log₁₀ CFU mL⁻¹ after a total dose of 288 J cm⁻², respectively.

Comparative inactivation results expressed as germicidal efficiencies

The inactivation capability of 405-nm light can be quantified as the germicidal efficiency (η), defined as the log₁₀ reduction of a bacterial population by inactivation per unit dose in joules per square centimeter (Wang *et al.*, 2005) as in Equation 2:

$$\eta = \log_{10}(N/N_0)/J \text{ cm}^2 \quad (2)$$

where N₀ = the bacterial starting population, and N = the final bacterial population after exposure. Percentage uncertainty of the mean germicidal efficiency value was determined by calculating the germicidal efficiencies for the lowest and highest log₁₀ reductions achieved at the final light dose applied for each microorganism, and the difference expressed as a percentage of the mean value.

Table 2 details the inactivation parameters, including germicidal efficiencies, for *C. jejuni*, *S. enteritidis*, and *E. coli* O157:H7 following exposure to high-intensity 405-nm light. The high sensitivity of *C. jejuni* to 405-nm light, relative to *S. enteritidis* and *E. coli* O157, is clearly demonstrated by the much higher germicidal efficiency value shown for *C. jejuni* in Table 2.

Discussion

The results of this study have demonstrated that high-intensity 405-nm light is highly bactericidal to *C. jejuni* and that *C. jejuni* is much more sensitive to 405-nm light than *S. enteritidis* and *E. coli* O157:H7. The dose required to inactivate both *S. enteritidis* and *E. coli* O157:H7 by 3 log₁₀ and 5 log₁₀ CFU mL⁻¹, respectively, was 288 J cm⁻², 18 times the dose required for a 5 log₁₀ CFU mL⁻¹ reduction to nondetectable levels (<1 CFU mL⁻¹) in *C. jejuni* (18 J cm⁻²). This difference in susceptibility to 405-nm light exposure is evident when the germicidal efficiencies of the three bacteria are compared, with *C. jejuni* having a germicidal efficiency value of 0.30, whereas *S. enteritidis* and *E. coli* O157:H7 have germicidal efficiency values of 0.02 and 0.01, respectively.

Macleane *et al.* (2009) quantified the germicidal efficiencies of a range of medically important bacteria exposed to 405-nm light and found that in general the Gram-negative bacteria tested (*Acinetobacter*, *Proteus*, *Pseudomonas*, *Klebsiella*, and *Es-*

cherichia species) had germicidal efficiency values of 0.04 or less. This concurred with the results for the 405 nm inactivation of *S. enteritidis* and *E. coli* O157:H7 in the present study. It is clear from these various results that *C. jejuni* is much more sensitive to and thereby much more rapidly inactivated by 405-nm light than a wide range of other Gram-negative bacteria.

Inactivation through 405-nm light exposure is thought to be through the generation of ROS, and microaerophilic *Campylobacter* spp. are particularly susceptible to stress from exposure to toxic oxygen species. *C. jejuni* has fewer stress regulators than both *S. enteritidis* and *E. coli*, possibly explaining the susceptibility of *Campylobacter* to ROS (Murphy *et al.*, 2006). It is anticipated that other *Campylobacter* species such as *Campylobacter coli*, another significant contributor to food-related illness (Tam *et al.*, 2003), will have a similar sensitivity to 405-nm light, as similar inactivation kinetics between species within the same genus has previously been demonstrated (Macleane, 2006; Macleane *et al.*, 2009).

Interestingly, some studies have shown evidence that exposure of *Campylobacter*-specific media to light and air causes production, in the media, of toxic oxygen species such as superoxide, hydroxyl radicals, singlet oxygen, and hydrogen peroxide (Bolton *et al.*, 1984; Juven and Rosenthal, 1985; Moran and Upton, 1987). Apparently, reversal of toxicity cannot occur once toxic oxygen compounds have been formed in the media (Bolton *et al.*, 1984; Juven and Rosenthal, 1985). A study by Bolton *et al.* (1984) has shown that these toxic oxygen species are highly bactericidal to *C. jejuni* causing inactivation of >5 log₁₀. Studies into the effect of light and air on *Campylobacter*-specific media have also found that the toxic species formed cause *Campylobacter* to shift to what was considered to be variable but non-culturable (VBNC) morphology (Moran and Upton, 1987). Whether or not exposure to 405-nm light might cause some conversion of *C. jejuni* cells to a possible VBNC state is out with the scope of the current study and further investigation would be required to elucidate this.

It is evident that studies on the effects of light on *Campylobacter* must take account of confounding factors such as possible indirect toxicity effects caused by the formation of toxic compounds in suspending liquids or culture media. In this context, blood-containing media, as used in the present study, are not subject to light-induced toxicity as any toxic oxygen species formed are quenched by detoxifying enzymes present in the media such as peroxidase, catalase, and superoxide dismutase (Bolton *et al.*, 1984; Moran and Upton, 1987). In addition to our use of blood agar for plate culture, previous experiments within our laboratory have shown that PBS, used to suspend *C. jejuni* during 405-nm light exposure,

develops no toxicity upon exposure to air and light, including high-intensity 405-nm light. Accordingly, we believe that the light inactivation of *C. jejuni* observed in the present study is due to the direct effect of 405-nm light on the exposed bacteria, an inactivating effect that is most probably associated with the production of ROS through the photostimulation of endogenous porphyrin molecules as has been proposed for other bacteria (Ashkenazi *et al.*, 2003; Hamblin *et al.*, 2005; Guffey and Wilborn, 2006; Maclean *et al.*, 2008a).

Photodynamic inactivation (PDI) of the bacteria in this study was achieved solely through 405-nm light exposure. As with the majority of the previous work into PDI of microorganisms, previous PDI studies of *Campylobacter*, *Escherichia*, and *Salmonella* spp. have involved the use of exogenous photosensitizing agents, which are excited into inducing ROS production upon exposure to visible light. Buchovec *et al.* (2009) showed that 5-aminolevulinic acid used in conjunction with 400-nm LEDs could inactivate *Salmonella enterica* by $6 \log_{10}$ CFU mL⁻¹, and Rovaldi *et al.* (2000) used an ϵ -pentyllysine conjugate and a laser of center wavelength 662 nm to inactivate *Campylobacter rectus* by around $7 \log_{10}$ CFU mL⁻¹. Alves *et al.* (2009) used Tri-Py⁺-Me-PF porphyrin as a photosensitizing agent for the inactivation of *E. coli*, achieving a $7 \log_{10}$ CFU mL⁻¹ reduction in *E. coli* numbers. Due to differences in the photosensitizer, light source, and peak wavelength of light used, it is difficult to compare data from these PDI studies; however, it is evident that addition of the exogenous photosensitizing agents resulted in more rapid inactivation of the bacteria compared to the results of the current study.

The use of UV light technologies for inactivation of a range of foodborne microorganisms, including *Campylobacter*, has been studied widely (Isohanni and Lyhs, 2009; Chun *et al.*, 2010); however, a significant limitation of UV light for decontamination of foods and contact surfaces is the poor penetration of UV light into solids and opaque liquids. Due to the longer wavelength of 405-nm light compared with UV light, 405-nm light has increased penetrability (Moan, 2001; Alexiades-Armenakas, 2006).

Exposure to UV irradiation is harmful to humans, causing ocular disease and skin cancers, and as such, must be used in a contained manner. Although high-intensity 405-nm light is not as germicidally efficient as UV light, its ease of operation and safety-in-use present distinct advantages as a decontamination technology; however, further investigations will be required to optimize bactericidal efficiency and to develop larger-scale systems for specific decontamination applications.

Overall, this study has demonstrated that *C. jejuni* and other enteric pathogens are inactivated by exposure to high-intensity 405-nm light. With further work, this inactivation method could potentially be used in conjunction with other food safety procedures for decontamination. The current study results indicate that this method may have particular use for the enhanced control of the highly sensitive *C. jejuni*, thereby potentially contributing to a reduction of transmission of this ubiquitous pathogen.

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Disclosure Statement

No competing financial interests exist.

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