International Journal of Food Microbiology 159 (2012) 267–273

Contents lists available at SciVerse ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Susceptibility of *Campylobacter* to high intensity near ultraviolet/visible 395 ± 5 nm light and its effectiveness for the decontamination of raw chicken and contact surfaces

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ARTICLE INFO

Article history:
Received 27 June 2012
Received in revised form 31 August 2012
Accepted 11 September 2012
Available online 19 September 2012

Keywords:
Food safety
Campylobacter
High intensity near ultraviolet/visible (NUV–vis) light
Decontamination
Chicken
Contact surfaces

ABSTRACT

Campylobacter is an important cause of human gastroenteritis worldwide. Chicken meat is frequently contaminated with this organism and is considered to be a significant source of infection. It has been predicted that lowering the numbers of *Campylobacter* on chicken meat can reduce the risk to public health. The aims of the current study were to investigate the susceptibility of *Campylobacter* to high intensity near ultraviolet/visible (NUV–vis) 395 ± 5 nm light and to examine its potential for the microbiological decontamination of raw chicken and contact surfaces. Exposure of *Campylobacter jejuni* and *Campylobacter coli* to NUV–vis light of irradiances was assessed at three distances (3, 12 and 23 cm) from the light source for up to 10 min, corresponding to doses of 0.06 to 18 J/cm^2. Overall, levels of inactivation in liquid and on raw chicken improved with longer exposure times and shorter distances from the light source. Reductions of more than 7 $\log_{10}$ CFU/mL were achieved for *Campylobacter* isolates in liquid following 2 min exposure at 3 cm. Exposure of skinless chicken fillet to NUV–vis light for 1 or 5 min at 3 cm distance reduced *C. jejuni* by 2.21 and 2.62 $\log_{10}$ CFU/g, respectively. Increasing the treatment time to 10 min did not significantly increase the level of inactivation. In general, NUV–vis light treatment did not affect the colour of raw chicken. Excluding treatments which resulted in excessive heating (>50 °C) of chicken skin, a maximum reduction of 0.95 $\log_{10}$ CFU/g was achieved for *C. jejuni* following 10 min exposure to NUV–vis light at 12 cm ($P<0.05$). For Enterobacteriaceae and total viable counts, significant reductions were achieved only on chicken fillet samples. Light treatments were significantly effective for decontaminating contact surfaces as there were no *C. jejuni* recovered from stainless steel or cutting board surfaces after NUV–vis light treatments from an initial inoculum of 2–4 $\log_{10}$ CFU/cm^2 ($P<0.05$). The current study demonstrates potential for the use of NUV–vis light for the inactivation of *Campylobacter* spp. in liquids, on raw chicken and contact surfaces. The incorporation of this technology could be implemented in a commercial processing plant at various stages, for example to decontaminate carcasses during air chilling. It could also be applied at critical stages within the plant to control microbial contamination on equipment surfaces.

1. Introduction

Campylobacter is the leading cause of human gastroenteritis worldwide and chicken meat is recognised as a significant source of infection (Zilbauer et al., 2008). In the EU, more than two thirds of market age broilers are colonised with *Campylobacter*, while over three quarters of carcasses are contaminated with this organism (European Food Safety Authority, 2010). Birds in commercial broiler flocks are frequently colonised by campylobacters at the time of slaughter and carcasses can also become contaminated during processing. These pathogens may survive subsequent stages of the food chain thereby presenting a

Abbreviations: NUV–vis, near ultraviolet/visible (light); ENT, Enterobacteriaceae; TVC, total viable counts; EFSA, European Food Safety Authority; DAFM, Department of Agriculture, Food and the Marine; ROS, reactive oxygen species; LED, light-emitting diode; FWHM, full-width at half maximum; UV, ultraviolet (light); HILP, high intensity light pulses; MRD, maximum recovery diluent; MHB, Mueller Hinton Broth; mCCDA, modified Charcoal Cefoperazone Deoxycholate Agar; CBA, Colombia Blood Agar; ΔL, change in colour (lightness); Δa, change in colour (redness); Δb, change in colour (yellowness).

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http://dx.doi.org/10.1016/j.ijfoodmicro.2012.09.006

risk to public health. Interventions aimed at reducing the concentrations on raw meat, have been identified in quantitative risk assessment models as effective public health interventions (Nauta et al., 2009). Quantitative microbial risk assessments have indicated that even moderate reductions in the numbers of campylobacters on carcasses (>1 $\log_{10}$ per carcass) can significantly reduce the risk of infection in humans and would result in a substantial decline in the incidence of human disease (Lindqvist and Lindblad, 2008).

Technologies such as ultraviolet light (UV) and high intensity light pulses (HILP) have demonstrated potential for the decontamination of raw chicken and other meats (Lyon et al., 2007; Chun et al., 2009; Isohanni and Lyhs, 2009; Keklik et al., 2009; Chun et al., 2010; Haughton et al., 2011a, 2011b). Although 254 nm UV light is reported to possess the greatest germicidal efficiency (Bintsis et al., 2000), recent studies have investigated the use of light from the near UV region (i.e. 390 nm) to visible light (≥400 nm) (Enwemeka et al., 2008; Maclean et al., 2008a, 2008b; Lipovsky et al., 2009; Murdoch et al., 2010). Although the germicidal efficiency may be lower than UV, visible light at a wavelength of 400 nm is considered to be a much safer alternative (Maclean et al., 2008a).

While UV light targets the DNA, the mechanism of inactivation by visible light is by an oxygen dependent process accredited to the photostimulation of exogenous porphyrin molecules which cause energy transfer and production of ROS that are bactericidal (Maclean et al., 2008a; Lipovsky et al., 2009). Visible light with a wavelength of 405 ± 5 nm has been found to have bactericidal activity against bacterial pathogens including methicillin resistant *Staphylococcus aureus* (MRSA) (Enwemeka et al., 2008; Maclean et al., 2008b). To date, only a single study has investigated the effect of high intensity 405 nm light on *Campylobacter jejuni* which was reported to be markedly more sensitive than *Escherichia coli* O157:H7 and required up to 16 times the dose applied to *C. jejuni* to achieve a 5 $\log_{10}$ CFU/mL reduction when suspended in buffered peptone water (Murdoch et al., 2010). The apparent susceptibility of *C. jejuni* to visible light warrants further research into its potential applications for the control of this organism.

The aims of the current study therefore were to investigate the susceptibility of a collection of 10 *Campylobacter* isolates (*C. jejuni* and *C. coli*) in liquid to NUV–vis light and to investigate its potential for the decontamination of raw chicken and food contact surfaces.

2. Materials and methods

2.1. Microorganisms, growth conditions and preparation of bacterial cultures

A total of ten *Campylobacter* isolates (seven *C. jejuni* and three *C. coli*) were assessed for their susceptibility to NUV–vis light in a transparent liquid matrix (maximum recovery diluent or MRD). The *C. coli* isolates were 1140 DF, 1662 DF and 2124 GF, while 323 BC, 1135 DF, 1136 DF, 1146 DF, 1147 DF, 1354 DF, and NCTC 11168 were *C. jejuni*. All campylobacters were isolated from retail chicken with the exception of 323 BC, and the typed *C. jejuni* strain, NCTC 11168, both of which were of human clinical origin. All *Campylobacter* chicken isolates were recovered and confirmed using methods described previously (Wang et al., 2002; Whyte et al., 2004). Frozen stock cultures of *Campylobacter* were maintained in defibrinated horse blood (Oxoid, Basingstoke, UK). Resuscitation of *Campylobacter* isolates was performed by inoculating a loopful of the thawed stock into Mueller Hinton Broth (MHB) (Oxoid) containing *Campylobacter* growth supplement (Oxoid) and incubated microaerobically for 48 h at 42 °C. A microaerobic atmosphere of 5% O_2 and 15% CO_2 was created using a GENbox jar and GENbox microaer gas generators (BioMérieux, Marcy l'Etoile, France). The enriched *Campylobacter* cultures were then streaked onto both Colombia Blood Agar (CBA) (Oxoid) and modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) (Oxoid), and incubated microaerobically for a further 48 h at 42 °C. *Campylobacter* suspensions were prepared by transferring a single colony from a 48 h blood agar plate to 20 mL of MHB containing *Campylobacter* growth supplements and incubated microaerobically at 42 °C for 24 h. The 24 h suspensions were centrifuged for 10 min at 30,000×*g* and the resulting pellet was washed and re-centrifuged twice in MRD before final resuspension in 20 mL MRD. This resulted in a liquid cell suspension of approximately 7 $\log_{10}$ CFU/mL.

2.2. High intensity NUV–vis light unit

The NUV–vis light was produced by a light-emitting diode (LED) array (OD-2049) (Opto Diode Corp, sourced from AP Technologies, Bath, UK) with a centre wavelength of 395 ± 5 nm, a bandwidth of 12 nm full-width at half maximum (FWHM) and a half intensity beam angle of 30° (Fig. 1). The irradiance (W cm^{-2}) of light emitted from the LED unit was measured using a UV–VIS Radiometer (model no. RM12, Dr. Gröbel UV Electronik, GmbH, Ettlington, Germany) fitted with an RM UV-A sensor (part no. 811030, Dr. Gröbel UV Electronik). Distances of 3, 12 and 23 cm were chosen for treatments with corresponding energy intensities presented in Table 1. These distances represented the most extreme to the least extreme treatments that were possible with the equipment used in the current study.

The light array was attached to a heat sink and cooling fan to dissipate the heat from the array. A fridge was used to house the equipment and a shelf constructed from polymethyl methacrylate was used to mount the unit. The array was mounted in the centre of the shelf, which included a number of circular holes to allow for air circulation and cooling of the LED unit. For safety, the door was fitted with an interlock to cut off the power supply to the light if the door was opened. The interlock did not affect the power supply to the fan allowing for continuous cooling of the LED unit.

2.3. Inactivation of Campylobacter in MRD by high intensity NUV–vis light

All 10 *Campylobacter* isolates (seven *C. jejuni* and three *C. coli*) were initially assessed for susceptibility to NUV–vis light in a liquid matrix (MRD) and the least susceptible isolate (1136 DF) was then selected for further study. *C. jejuni* and *C. coli* pure bacterial cultures (~7 $\log_{10}$ CFU/mL) were prepared as described in Section 2.1. Samples (3 mL) were placed in petri dishes of 50 mm diameter (Sterilin Limited, Caerphilly, United Kingdom), resulting in a liquid depth of 1.5 mm and exposed to NUV–vis light at various distances (3, 12 and 23 cm) and exposure times. Sample temperatures were measured pre- and post-treatment using a K-type thermocouple attached to a Grant Data Logger (Squirrel 2040, Grant Instruments, Cambridge, United Kingdom), which indicated that the sample temperatures did not exceed 30 °C. Experiments were carried out in triplicate.

2.4. Decontamination of raw chicken by NUV–vis light

To investigate the effectiveness of NUV–vis light for the decontamination of raw chicken, skinless chicken breast meat and chicken skin were inoculated with the least susceptible *C. jejuni* isolate (*C. jejuni* 1136 DF) as determined by earlier susceptibility studies, and treated with 395 ± 5 nm light. Pieces of raw skinless breast meat and skin were dipped in the 24 h pure bacterial suspension, as described previously, for 20 s. The inoculated skinless breast meat and skin sections were then placed in sterile petri dishes and stored at ambient temperature for 30 min to allow bacterial attachment before samples were exposed to NUV–vis light at distances of 3, 12 and 23 cm, for 1, 5 and 10 min. Skin samples were treated on both sides, which involved treating the upper surface and then aseptically inverting and transferring to a separate sterile petri dish for further 395 nm light treatment. Sample temperatures were monitored at 1 s intervals during treatments by inserting a K-type thermocouple into the upper surface of