LWT - Food Science and Technology 68 (2016) 579–588

Contents lists available at ScienceDirect

LWT - Food Science and Technology

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

Gene expression analysis for *Listeria monocytogenes* following exposure to pulsed light and continuous ultraviolet light treatments

Aaron R. Uesugi, Lillian C. Hsu, Randy W. Worobo**, Carmen I. Moraru*

Department of Food Science, 251 Stocking Hall, Cornell University, Ithaca, NY 14853, USA

ARTICLE INFO

Article history:
Received 8 July 2015
Received in revised form
30 December 2015
Accepted 4 January 2016
Available online 7 January 2016

Keywords:
Pulsed light
Continuous UV
Listeria monocytogenes
Gene expression

ABSTRACT

Pulsed Light (PL) is effective for inactivation of bacteria in food and non-food systems, yet the mechanisms of inactivation are not fully understood. In this work, the response of *Listeria monocytogenes* to PL and continuous ultraviolet light (UV) was investigated using whole genome DNA microarray analysis. Suspensions of *L. monocytogenes* were treated with 3.20 J/cm^2 of PL and 33 mJ/cm^2 of UV, respectively, which yielded comparable levels of inactivation. In a separate experiment, cells were exposed to 3.20 J/cm^2 of full spectrum PL and UV-blocked PL. Whole genome microarray analysis of *L. monocytogenes* was performed to identify differential gene expression after the treatments, using a 1.5 fold change cutoff and adjusted $P < 0.05$. Compared to untreated cells, PL and UV treated cells had higher transcription levels for 80 and respectively 39 multiple stress related proteins, motility genes, and transcriptional regulators. Exposure to UV-blocked PL resulted in 131 motility related genes and cell membrane related genes with lower transcription levels, and no genes with higher transcription levels compared to untreated cells. The data suggests that the microbicidal effects of PL were primarily due to UV, and no significant differences in gene expression between PL and UV treated cells were found.

1. Introduction

Pulsed Light (PL) technology is a light-based alternative to thermal treatment for killing microorganisms in food and non-food systems, which has been considered by some to be more potent than continuous ultraviolet light (UV) treatment. UV uses germicidal mercury lamps that emit light at 254 nm, while PL uses gas (Xenon) discharge lamps that emit short, intense pulses of light that includes UV-C (200–280 nm), UV-B (280–315 nm), UV-A (315–400 nm), visible (VIS) (400–700 nm), and near infrared (NIR) light (700–1100 nm).

Microbial inactivation in UV treatment is caused by the formation of pyrimidine dimers between adjacent bases in the DNA molecule (Bintsis, Litopoulou-Tzanetaki & Robinson, 2000). There is strong evidence that UV is the main contributor to PL inactivation of microorganisms (Woodling & Moraru, 2007), but other type of cellular damage has also been reported. Takeshita et al. (2003) reported increased protein elution, including enlarged vacuoles, cell membrane distortion, and shape changes in *Saccharomyces cerevisiae* cells treated with PL. Wekhof, Trompeter & Franken (2001) showed *Aspergillus niger* spores with ruptured tops, reportedly caused by steam escaping from the overheated spores. Thermal effects in PL treatment have also been reported by Ozer and Demirci (2006), who observed significant heating at the substrate level, at very high PL doses (180 pulses of light, at 3 cm from the lamp). Cheigh, Park, Chung, Shin, and Park (2012) compared transmission electron micrographs of untreated, PL treated, and continuous UV treated *Listeria monocytogenes* and *Escherichia coli* O157:H7 cells, and found that the membranes of cells untreated or treated with continuous UV remained intact. In contrast, membranes of PL treated cells were significantly disrupted, internal organization was lost and cellular components leaked out of the cells. In this case, too, cells were exposed to extended PL treatments (180 s), which are not typical for commercial PL treatments. In the US, the FDA limits PL doses to 12 J/cm^2 (FDA, 1996), while the treatments reported in these two studies used doses several fold higher than this limit. Significant structural effects at low germicidal doses of PL have not been reported. Therefore, questions still remain regarding the mechanism of microbial inactivation by PL, particularly if any significant differences exist in the mechanism of

* Corresponding author.
** Corresponding author. 360 Stocking Hall, Cornell University, Ithaca, NY 14853, USA.
E-mail addresses: rww8@cornell.edu (R.W. Worobo), cim24@cornell.edu (C.I. Moraru).

http://dx.doi.org/10.1016/j.lwt.2016.01.007
0023-6438/

inactivation by PL vs continuous UV.

This work aims to address these questions by looking beyond inactivation data, and focusing on the effects of the two treatments on bacterial cells exposed to continuous UV and PL. Specifically, the objective of this work was to investigate and compare the global transcriptional response of *L. monocytogenes* cells exposed to PL as compared to continuous UV. Additionally, gene expression effects caused by exposure to the VIS and NIR spectrum of PL were also investigated. This will generate further evidence on the role of different spectral components in microbial cell death, and help us gain further understanding of the mechanisms of inactivation in PL treatment. *L. monocytogenes*, a psychrotrophic Gram positive, nonspore-forming, facultative anaerobic foodborne pathogen with high mortality rate (Mead et al., 1999), was chosen as a challenge microorganism. This pathogen was frequently used in PL inactivation studies due to its importance for food safety. Inactivation levels for *L. monocytogenes*, at FDA approved doses (below 12 J/cm^2), range from 1.5 log CFU in ready-to-eat cured meats (Ganan, Hierro, Hospital, Barroso, & Fernandez, 2013), to about 2.5 log CFU in biofilms (Montgomery & Banerjee, 2015) or on the surface of American cheese (Can, Demirci, Puri, & Gourama, 2014), and up to 3 log reduction on apple slices (Ignat, Manzocco, Maifreni, Bartolomeoli, & Nicoli, 2014). In addition, the genome of *L. monocytogenes* is very well studied, which is particularly significant for this work.

2. Materials and methods

2.1. Bacterial strain and growth conditions

L. monocytogenes serotype 1/2a strain 10493S (Bishop & Hinrichs, 1987) from the Food Microbiology and Safety Laboratory at Cornell University (Ithaca, NY) was used. For each replicate, *L. monocytogenes* was streaked onto Brain Heart Infusion (BHI; Difco, Sparks, MD) from glycerol stock cultures stored at −80 °C, then incubated at 37 °C for 24 h. Cultures were maintained on slants of Tryptic Soy Agar (TSA; Difco, Sparks, MD) for up to 3 months at 4 °C. Stock culture was streaked to TSA plates for isolation and incubated at 37 °C for 24 h. A single colony was subsequently inoculated into 5 mL Defined Medium (DM) for *L. monocytogenes* (Premaratne, Lin & Johnson, 1991) and incubated for 12–18 h at 37 °C with shaking (225 RPM). Fifty μL of the culture was transferred to 5 mL of fresh pre-warmed (37 °C) DM and incubated at 37 °C with shaking (225 RPM) until an optical density at 600 nm (OD_{600}) of 0.4 (early log phase). One mL culture was then transferred to 100 mL of pre-warmed (37 °C) DM and incubated at 37 °C with shaking (225 RPM) until an OD_{600} = 1.0 plus 3 h was reached (early stationary phase).

2.2. PL and UV treatments

Fifteen mL inoculum were transferred into a Petri plate of 100 mm diameter. This was placed onto a MS 3 basic orbital shaker (IKA Works, Wilmington, NC), wrapped in aluminum foil to avoid light absorption, and set to 500 RPM to generate turbulence and ensure uniform light exposure.

PL treatments were conducted with a RS-3000C SteriPulse System (Xenon Corporation, Woburn, MA), equipped with a Xenon flash lamp, which delivers 3 pulses per second (pulse width of 360 μs). The treatment dose (fluence) was measured (in triplicate) using a PE25BBH pyroelectric head with Nova II display (Ophir Optronics Inc. Wilmington, MA), placed in the center of the treatment chamber, at the same distance from the lamp as the samples. The detector's surface was covered with a stainless steel aperture cover with a 1 cm^2 circular opening.

For exposure to UV-blocked PL, a filter that only allowed the transmission of light with λ > 400 nm was placed inside the PL unit, at 5 mm from the lamp (Woodling & Moraru, 2007). More light pulses were necessary to reach the same fluence for UV-blocked PL compared to full spectrum PL: a fluence of ~3.2 J/cm^2 required 12 pulses of blocked-UV PL vs. 6 pulses of full spectrum PL.

For UV treatments, a germicidal lamp (254 nm) located inside a NuAire 425 biological safety cabinet (NuAire Laboratory Equipment Supply; Plymouth, MN) was used. UV treatments lasted up to 120 s, corresponding to a dose of 33 mJ/cm^2. For the control, the inoculum was placed on a shaker for 120 s, with the UV lamp turned off. UV fluence measurements were made in triplicate using a UVX radiometer (Ultra-Violet Products; Upland, CA).

As negative control, *L. monocytogenes* cells not exposed to light treatments were used.

2.3. Total RNA isolation

After each treatment, 1 mL sample was used for cell enumeration. Dilutions were performed in Butterfield's Phosphate Buffer; 100 μL was spread plated in duplicate onto TSA and incubated for up to 48 h at 37 °C. The remaining cell culture was transferred to an Al foil wrapped centrifuge tube and placed in an incubator at 37 °C, with shaking (225 RPM) (Innova 43 shaker, New Brunswick Scientific), for 5 min, after which cells were harvested and used for RNA isolation, following the procedure described in the Supplementary Material. After extraction, RNA (purified or unpurified) not used immediately was stored at −80 °C. Three biological replicates were performed for each experimental condition.

2.4. Microarray construction

Whole-genome microarrays were designed with 70-mer oligonucleotides representing all 2857 ORFs identified in the genome sequence of *L. monocytogenes* EGD-e (Gilot, Jossin, & Content, 2000 The Array-Ready Oligo sets for 2857 ORFs from *L. monocytogenes* EGD-e and the 70-mer oligonucleotides were purchased from Operon Biotechnologies (Huntsville, AL). An *ilnD* probe was also designed using Array OligoSelector (http://arrayoligosel.sourceforge.net/), based on the *inlD* sequence for *L. monocytogenes* 10403S, since *inlD* is not present in strain EGD-e (Gilot et al., 2000). Probes targeting five *S. cerevisiae* genes were used as non-hybridizing controls, as described by McGann, Ivanek, Wiedmann, & Boor (2008). Salmon sperm DNA and serial dilutions of chromosomal *L. monocytogenes* 10403S DNA were spotted on the glass array for quality control and signal normalization. Since *L. monocytogenes* strains 10403S and EGD-e represent the same *L. monocytogenes* lineage (II), serotype (1/2a), and ribotype (DUP-1039C) (Wiedmann et al., 1997), probes designed using the EGD-e genome were expected to hybridize well with 10403S genes.

The controls and 70-mer-oligonucleotides were spotted in duplicate on UltraGAPS slides (Corning, NY) using a XYZ arrayer from the Cornell Microarray Core Facility (Ithaca, NY). The slides were UV cross-linked (300 mJ for 1 min) to immobilize the oligonucleotides to their surface and then stored in a desiccator protected from light, at room temperature, until use.

2.5. cDNA labeling and competitive microarray hybridization

For each RNA sample, 6 μg of total RNA was reverse-transcribed using Superscript III RT (Invitrogen), as described in the Supplementary Material. The resulting cDNA was dried completely and labeled with the appropriate Cy dye, and microarray slides were prepared as described in the Supplementary Material. Within