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

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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/cm² of PL and 33 mJ/cm² of UV, respectively, which yielded comparable levels of inactivation. In a separate experiment, cells were exposed to 3.20 J/cm² 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.

Keywords: Pulsed Light; continuous UV; *Listeria monocytogenes*; gene expression

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 & 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 & 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 et al. (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/cm² (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 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 *Listeria 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 & 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/cm²), range from 1.5 log CFU in ready-to-eat cured meats (Ganan et al., 2013), to about 2.5 log CFU in biofilms (Montgomery and Banerjee, 2015) or on the surface of American cheese (Can et al., 2014), and up to 3 log reduction on apple slices (Ignat et al., 2014). In addition, the genome of *L. monocytogenes* is very well studied, which is particularly significant for this work.

Materials and Methods

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 (OD₆₀₀) 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 OD₆₀₀ = 1.0 plus 3h was reached (early stationary phase).

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 cm² circular opening.

For exposure to UV-blocked PL, a filter that only allowed the transmission of light with $\lambda > 400$ 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/cm² 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/cm². 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.

Total RNA isolation

After each treatment, 1 mL sample was used for cell enumeration. Dilutions were performed in Butterfield's Phosphate Buffer; 100 μ L 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.

Microarray construction

Whole-genome microarrays were designed with 70-mer oligonucleotides representing all 2,857 ORFs identified in the genome sequence of *L. monocytogenes* EGD-e (Gilot, Jossin & Content, 2000). The Array-Ready Oligo sets for 2,857 ORFs from *L. monocytogenes* EGD-e and the 70-mer oligonucleotides were purchased from Operon Biotechnologies (Huntsville, AL). An *inlD* 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, Jossin & Content, 2000). Probes targeting five *Saccharomyces cerevisiae* genes were used as non-hybridizing controls, as described by McGann & al (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.

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 1 h from preparation, slides were scanned with a GenePix 4000B scanner (Molecular Devices, Sunnyvale, CA) at the Cornell University

Microarray Core Facility. Microarray experiments were performed using RNA from three biological replicates.

Microarray data analysis

Raw TIFF images were analyzed using GenePix Pro 6.0 software, using the methodology described in the Supplementary Material. Statistical significance of differential expression results was assessed based on P values, which were adjusted for multiple comparisons by controlling for the false discovery rate. Genes with adjusted $P < 0.05$ were considered statistically significant. A change of 1.5 fold was used as minimum threshold for identification of differentially expressed genes.

Results and Discussion

Inactivation of *L. monocytogenes* by UV, PL, and UV-blocked PL treatments

PL and UV inactivation curves for *L. monocytogenes* are shown in Figure 1. PL resulted in a relatively linear reduction over the fluence range used. Previous studies have shown tailing in PL inactivation, at fluence levels of up to 12 J/cm^2 (Uesugi, Woodling & Moraru, 2007). UV inactivation curves showed an initial shoulder, followed by a relatively linear inactivation curve.

To directly compare the transcriptional response of cells exposed to the two treatments, it was important to achieve the same level of microbial kill. Based on the inactivation curves, a fluence of 3.20 J/cm^2 (6 pulses) for PL and 33 mJ/cm^2 (120 s) for UV were chosen, which resulted in reductions of *L. monocytogenes* of 3.10 log CFU for PL and 3.14 log CFU for UV, respectively. When using these doses in the microarray study, slightly different inactivation levels were obtained: $2.33 \pm 0.36 \text{ log CFU}$ for PL, and $3.06 \pm 0.60 \text{ log CFU}$ for UV (Table 1). These inactivation levels were not statistically different from each other.

After exposure to UV-blocked PL, at a similar fluence as for full spectrum PL (3.25 J/cm²), a slight microbial growth (0.14 log CFU) compared to the untreated control was obtained (Table 1), consistent with prior observations (Woodling & Moraru, 2007).

Global gene expression analysis

Eighty genes from *L. monocytogenes* cells exposed to PL and 39 genes from *L. monocytogenes* cells exposed to UV, representing 2.8 and 1.4 % of the 2,857 ORF's in the array, respectively, showed higher transcription levels (≥ 1.5 fold change, adjusted $P < 0.05$) in early stationary phase cells grown at 37 °C (Table 2). No increase in transcription was observed after treating *L. monocytogenes* with UV-blocked PL, although 131 (4.6%) genes showed lower transcription levels compared to untreated cells (Table 3). No genes showed decreased transcription levels compared to untreated cells following either PL or UV treatments. Overall, the results show that: (i) a number of *L. monocytogenes* genes were differentially expressed following exposure to PL or UV; and (ii) a number of *L. monocytogenes* genes had lower transcription levels after exposure to UV-blocked PL.

Genomic expression of L. monocytogenes following exposure to PL, UV and UV-blocked PL

Stress response genes

The *L. monocytogenes* genome contains a number of stress-response genes to allow for its survival under various environmental conditions, including low temperature (Helmann & al., 2001; Chan, Raengpradub, Boor & Wiedmann, 2007), varying pH (Ferreira, O'Byrne & Boor, 2001), and osmolarity (Seeliger & Jones, 1986). For the PL treated cells, lmo1138, lmo2485, and lmo2748, and for the UV treated cells, lmo0609 and *sigL* (lmo2461) showed increased transcription levels. Both lmo2485 and lmo2748 are class II stress response genes, representing a general stress response \ regulated by the alternative sigma factor SigB (van der Veen & al.,

2007); however, the SigB encoding gene did not show increased differential expression in any of the treatments. The lmo2485 gene was previously identified as being SigB regulated in *L. monocytogenes* (Kazmierczak, Mithoe & Wiedmann, 2003). lmo1138, similar to *clp*, is a heat stress response gene that codes for an ATP dependent protease involved in the degradation of misfolded proteins (Kruger & al., 2000). For UV treated cells, lmo0609, whose function is similar to an *E. coli* phage shock protein, showed increased transcription. The phage shock protein operon is usually induced by phage infection, heat, osmotic stress, and ethanol (Model, Jovanovic, & Dworkin, 1997); therefore, its up-regulation after exposure to UV is expected. The gene *sigL* (lmo2461), which showed increased levels after UV exposure, is induced by a variety of stresses, such as low temperatures, organic acids, and high osmolarity (Raimann, Schmid, Stephan & Tasara, 2009).

However, it was surprising that the “dark repair” genes, *uvrA* (lmo2488), *uvrB* (lmo2489), and *uvrC* (lmo1234) did not show increased transcription. Likewise, there was no increase in *recA* (lmo1398), the major regulator of the SOS response involved in DNA repair and the resuming of replications that have stalled (Maul & Sutton, 2005). Expression of photolyase (lmo0588), an indicator for light repair of damaged DNA (Snyder & Champness, 2003), did not increase either, but this was expected since cells were kept under dark following treatment. The mode of action for UV light is DNA damage, and thus it would have been expected that the DNA damage and stress response related genes would be up regulated. A possible explanation for the observed results may be the fact that the UV fluence used in this study was insufficient to cause the upregulation of these genes in the majority of the microbial population, and therefore a significant change in gene expression could not be detected.

For cells exposed to UV-blocked PL, there were no stress response genes that exhibited

transcriptional changes compared to untreated cells, which agrees with the inactivation results.

Motility genes

The flagellin gene *flaA* (lmo0690) showed up-regulation following exposure to UV.

Flagella production is usually regulated by temperature, and at 37 °C there is little motility and flagella in *L. monocytogenes* (Way & al, 2004). *L. monocytogenes* shows maximum

transcription of *flaA* when grown at 22°C (Peel, Donachie & Shaw, 1988). The increased

transcription in *flaA* after 120 s exposure to UV was likely in response to the much longer

exposure time in UV compared to PL needed to obtain similar lethality (120 s for UV vs. 2 s for

PL). It is important to note that while the total duration of the PL treatment was 2 s, the actual

exposure time to PL lasted only a few milliseconds (6 pulses at 360 µs per pulse). It is likely that

during this very short treatment time cells may have not been able to express flagellin. The

different expression of flagellin is one of the few differences observed between PL and UV. For

cells exposed to UV-blocked PL, a down regulation in 16 genes related to flagella production

was observed. The difference in flagellar motility gene expression after the two treatments may

be attributed to temperature differences. Specifically, UV treated cells were at ambient

temperature for 2 min during the treatment, while the PL treated cells were at ambient

temperature for only 2 s. The longer time at ambient temperature may result in the up regulation

of flagellar motility genes, as has been reported by Gründling et al. (2004).

Transcription and translation genes

PL and UV treated cells expressed a total of 12 genes with increased regulation: 6 genes after PL treatment and 9 after UV treatment, with 3 common for the two treatments. lmo0770, a

gene with increased transcription in both the PL and UV treated cells, is similar to transcriptional

regulators in the *lacI* family, which act as inducers for the *lac* operon. The defined growth

medium used for *L. monocytogenes* in this study contained glucose as the carbohydrate source, so regulation of the *lac* operon would be expected to be tightly controlled. For the UV treated cells, there was also an increase in the repressor of myo-inositol operon *iolR* (lmo0382). Glucose was shown before to repress the expression of the transcripts of the myo-Inositol operon in *Clostridium perfringens* (Kawsar & al, 2004); therefore, increases of the repressor protein under the conditions used in this study (i.e. the defined growth medium) should be expected. A similar scenario was seen with lmo0575, a regulator of the GntR family, which is involved in gluconate repression (Tong, Porco, Isturiz & Conway, 1996).

Additionally, numerous undefined transcriptional regulators showed increased expression levels: lmo0266 and lmo1263 for both treatments; lmo0376, lmo0602, and lmo0822 for PL and lmo0815, lmo0873, and lmo1716 for UV. *L. monocytogenes* genes that were positively regulated included several related to a variety of phosphotransferase system (PTS) genes involved in regulation of glucose (*yeeI*, or lmo0369), fructose (lmo0631), beta-glucoside (lmo0875), pentitol (lmo1972), and cellobiose (lmo2780). Not including lmo0369, the other PTS related genes involve either enzyme IIA or IIB of the PTS system. Upregulation of this variety of PTS systems for multiple types of sugars may be an indication that the cells are under stress and up-taking as many sugars as possible for use as catabolites for possible cell repair. PTS operons have been suggested to play a role in carbon assimilation at low temperatures or exposure to stresses such as energy (Chan, Raengpradub, Boor & Wiedmann, 2007; Raengpradub, Wiedmann & Boor, 2008).

Cells treated with UV-blocked PL showed down-regulation of lmo2173, a protein similar to the sigma-54-dependent transcriptional activator (σ^{54}), which is primarily involved in the control of carbon metabolism by regulation of the phosphotransferase system (PTS) (Arous & al,

2004). The expression of the *mptACD* operon, as well as of several other PTS operons, appears to be under the direct control of σ^{54} . The *mptACD* operon encodes subunits of the PTS permease of the mannose family. This mannose regulation is consistent with the additional down-regulation of lmo0096, lmo0097, and lmo0098, which are similar to the PTS mannose specific system. Stoll & Goebel (2010) found that in the case of *L. monocytogenes* EGD-e, the PTS mannose permease encoded by lmo00096-98 is the primary glucose transporter, when glucose is the sole carbon source. Therefore, the down-regulation of these genes in this study, where glucose was the sole carbon source, was surprising and might indicate that in the absence of UV light, cellular uptake of glucose occurs by other mechanisms.

Cell membrane associated genes

The bacteria cell wall is a complex structure that provides the first defense of the cell to the environment and potential stresses; in case of pathogens, it also has components that aid in pathogenesis and host cell invasion. PL treatment induced up-regulation of several important virulence genes, including *inlH* (lmo0263), *sepA* (lmo2157), and lmo0721. InlH is a cell surface protein in the family of internalin related proteins that helps promote invasion of host cells (Schubert & al, 2001). SepA is a major extracellular protein of *Shigella*, found to be involved in tissue invasion (Benjelloun-Touimi, Sansonetti & Parsot, 1995), but its function in *L. monocytogenes* has not been confirmed. lmo0721 is a putative fibronectin-binding protein that helps cells bind to the surface of host cells (Gilot, Jossin & Content, 2000). Interestingly, after exposure to UV-blocked PL, lmo1634, alcohol-acetaldehyde dehydrogenase, was down-regulated by the largest fold change observed in this study (17.5). lmo1634 is an adhesion protein (LAP) (Kazmierczak, Mithoe, Boor & Wiedmann, 2003), which interacts with mammalian heat shock proteins to help initiate infection. The reason for up- and down-regulation

of proteins with similar functions after PL and UV-blocked PL treatments, respectively, is not clear. PL and UV treatments also increased regulation of other cell membrane proteins such as alkaline phosphatase (lmo1870) and a cation efflux transport protein (lmo2575). The commonality of these surface induced proteins is their involvement in signaling and interacting with the external environment. Efflux pumps often remove toxins from the cell and the other proteins can provide feedback to external factors that *L. monocytogenes* may encounter.

In cells treated with UV-blocked PL, several genes associated with cell growth were down-regulated, including those associated with peptidoglycan synthesis, *mugG* (lmo2035) and *murD* (lmo2036) (Mengin-Lecreulx & al, 1989), and cell wall synthesis, *mraY* (lmo2037) (Branstrom & al, 2000). The repression of genes related to cell growth and division was possibly due to the cells being in early stationary phase prior to exposure to the UV-blocked PL. Three genes involved with chemotaxis (lmo0683, lmo0689, lmo1699) also showed lower transcription levels. This further suggests that UV-blocked treatment did not induce stress response in cells.

Metabolic and unknown genes

Genes related to carbohydrate, amino acid, and nucleic acid metabolic pathways were up-regulated following exposure to PL and UV and down-regulated following treatment with UV-blocked PL. For PL and UV treated cells, an exonuclease (lmo1881) and an RNase (lmo1880) were also up-regulated. This may indicate the need to catabolize RNA that was generated as a stress response system, or that single-stranded DNA that was damaged needed to be degraded.

Conclusions

Numerous stress response, transcription/ translation, motility, and cell membrane genes were up-regulated following exposure to PL or UV, but no significant differences in the gene

types that were up-regulated after exposure to the two treatments were found. UV-blocked PL treatment showed down-regulated transcription/translation, motility, and cell membrane associated genes. This observation, corroborated with the fact that no inactivation of *L. monocytogenes* was observed when UV was removed from PL, provide strong evidence that the UV component of the PL spectrum was responsible for inactivation of *L. monocytogenes*, and that the visible and NIR portions of the spectrum, at the doses used in this study, did not lead to cell death.

Some slight differences in inactivation kinetics between PL and UV treatments, primarily the presence of a shoulder in the UV inactivation curve, were observed. While the maximum effectiveness of the two treatments has not been probed here, one important practical observation is that a much shorter treatment time is needed to achieve a similar inactivation level in PL compared to continuous UV, which recommends PL technology as a faster alternative to continuous UV for food processing applications.

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 459

Figure 1. Reduction of *L. monocytogenes* after exposure to Pulsed Light (A) and UV light (B).

465 **Table 1.** *L. monocytogenes* after exposure to PL, UV-blocked PL and continuous UV light.

Population in untreated Control (log CFU/ml)	Reduction (log CFU)		
	Pulsed Light	UV blocked Pulsed Light	Continuous UV Light
9.47 ± 0.54	-2.33 ± 0.36 ^a	0.14 ± 0.20 ^b	-3.06 ± 0.60 ^c

466

467 ^a Exposure to 3.20 J/cm²

468 ^b Exposure to 3.25 J/cm²

469 ^c Exposure to 33 mJ/cm²

470 **Table 2.** Genes identified by microarray analysis to be up-regulated following exposure to PL, UV Light, or UV blocked PL^a

Protein category and gene	Protein function ^b	Change (n-fold) in cells exposed to ^c :		
		PL	UV light (254 nm)	UV blocked PL
(1)	(2)	(3)	(4)	(5)
Stress				
lmo0609	Similar to <i>E. coli</i> phage shock protein E		1.6	
lmo1138	Similar to ATP-dependent Clp protease proteolytic component	1.6		
lmo2461 (<i>sigL</i>)	RNA polymerase sigma-54 factor (sigma-L)		1.7	
lmo2485	Similar to <i>B. subtilis</i> yvlC protein	1.6		
lmo2748	Similar to <i>B. subtilis</i> stress protein YdaG	1.7		
Transcription or Translation				
lmo0266	Similar to transcriptional regulators	1.8	2.3	
lmo0376	Similar to putative transcription regulator	1.6		
lmo0382	Similar to <i>B. subtilis</i> transcription repressor of myo-inositol catabolism operon IolR		1.7	
lmo0575	Similar to transcription regulator GntR family		1.6	
lmo0602	Weakly similar to transcription regulator	1.5		
lmo0770	Similar to transcriptional regulator (LacI family)	1.5	1.6	
lmo0815	Similar to transcription regulators		1.5	
lmo0822	Similar to transcriptional regulators	1.7		
lmo0873	Similar to transcriptional regulator (antiterminator)		1.6	
lmo1263	Similar to transcriptional regulator	1.7	1.5	
lmo1716	Similar to putative transcription regulators		1.6	

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(1)	(2)	(3)	(4)	(5)
Cell membrane function				
lmo0263 (<i>inlH</i>)	Internalin H	1.6		
lmo0593	Similar to transport proteins (formate?)	1.7		
lmo0721	Putative fibronectin-binding protein	1.5		
lmo0767	Similar to ABC transporter, permease protein	3.0		
lmo1697	Similar to putative transmembrane proteins	1.5		
lmo1870	Similar to alkaline phosphatase	1.5	1.5	
lmo2157 (<i>sepA</i>)	sepA	1.7		
lmo2575	Similar to cation transport protein (efflux)		1.8	
Phage related				
lmo0113	Similar to protein gp35 from Bacteriophage A118	1.6		
lmo2276	Similar to an unknown bacteriophage protein	1.5		
Motility				
lmo0690 (<i>flaA</i>)	Flagellin protein		1.8	
Phosphotransferase systems				
lmo0369	Conserved hypothetical protein, highly similar to <i>B. subtilis</i> YeeI protein		1.8	
lmo0631	Similar to PTS system, fructose-specific IIA component		1.5	
lmo0875	Similar to PTS system, beta-glucoside enzyme IIB component	1.5		
lmo1972	Similar to pentitol PTS system enzyme II B component		1.6	
lmo2780	Similar to cellobiose PTS enzyme IIA	1.5		
DNA/RNA metabolism				
lmo0213 (<i>pth</i>)	Similar to peptidyl-tRNA hydrolase		1.7	
lmo1096 (<i>guaA</i>)	Highly similar to GMP synthetase		1.5	
lmo1691	Similar to deoxyuridine triphosphate nucleotidohydrolases		1.5	
lmo1698	Similar to ribosomal-protein-alanine N-acetyltransferase	1.7		
lmo1880	Similar to similar to RNase HI	1.7		
lmo1881	Similar to 5-3 exonuclease		1.7	

(1)	(2)	(3)	(4)	(5)
Metabolism				
lmo0266	Similar to succinyldiaminopimelate desuccinylase	1.8		
lmo0359	Similar to D-fructose-1,6-biphosphate aldolase	1.5		
lmo0539	Similar to tagatose-1,6-diphosphate aldolase	1.6		
lmo0580	Weakly similar to carboxylesterase	1.6		
lmo0857	Similar to carboxylesterase	1.7		
lmo1051	Similar to formylmethionine deformylase and to <i>B. subtilis</i> YkrB protein	1.9		
lmo1091	Similar to glycosyltransferases	1.7	1.8	
lmo1180	Similar to putative carboxysome structural protein	1.6		
lmo1244	Weakly similar to phosphoglycerate mutase 1	1.5		
lmo1285	Conserved hypothetical protein, similar to <i>B. subtilis</i> YneT protein	1.5		
lmo1647	Similar to 1-acylglycerol-3-phosphate O-acyltransferases	1.7		
lmo1883	Similar to chitinases	1.7		
lmo2385	Similar to <i>B. subtilis</i> YuxO protein	1.5		
lmo2433	Similar to acetyesterase	1.5		
lmo2542	Similar to protoporphyrinogen oxidase	1.6		
lmo2674	Similar to ribose 5-phosphate epimerase	1.6		
lmo2830	Similar to thioredoxin	1.7		
Other or hypothetical proteins				
lmo0019	Unknown	1.6		
lmo0216	Highly similar to <i>B. subtilis</i> YabO protein		1.7	
lmo0254	Unknown	1.5		
lmo0267	Similar to other proteins	1.5	1.9	
lmo0267	Similar to other proteins			
lmo0310	Unknown	1.6	1.5	
lmo0310	Unknown			
lmo0321	Similar to unknown proteins	1.7		

(1)	(2)	(3)	(4)	(5)
lmo0377	Unknown		1.5	
lmo0397	Similar to unknown proteins		1.6	
lmo0515	Conserved hypothetical protein	1.6		
lmo0579	Similar to unknown protein	1.5		
lmo0628	Unknown	1.6		
lmo0629	Unknown		1.5	
lmo0654	Unknown	2.2	1.6	
lmo0661	Similar to unknown proteins		1.5	
lmo0758	Unknown	1.7	1.6	
lmo0771	Unknown	1.6	1.7	
lmo0800	Similar to <i>B. subtilis</i> YqkB protein	1.6		
lmo0903	Conserved hypothetical protein	1.8		
lmo0911	Unknown	1.6		
lmo0953	Unknown	2.0		
lmo1069	Similar to <i>B. subtilis</i> YlaI protein	1.5		
lmo1140	Unknown	1.7		
lmo1236	Similar to <i>B. subtilis</i> YslB protein	1.5		
lmo1245	Unknown	1.6		
lmo1312	Unknown	4.5		
lmo1468	Similar to unknown proteins	1.6		
lmo1515	Similar to unknown protein	1.5	1.6	
lmo1526	Similar to unknown proteins	1.8		
lmo1580	Similar to unknown protein	1.7		
lmo1612	Similar to unknown proteins		1.6	
lmo1670	Similar to conserved hypothetical proteins	1.7		
lmo1790	Similar to unknown proteins	1.6		
lmo1830	Similar to conserved hypothetical proteins	1.7		
lmo1888	Similar to hypothetical proteins	1.7		

(1)	(2)	(3)	(4)	(5)
lmo1919	Similar to unknown proteins	1.5		
lmo2158	Similar to <i>B. subtilis</i> YwmG protein	1.6		
lmo2177	Similar to unknown protein		1.8	
lmo2210	Unknown		1.6	
lmo2213	Similar to unknown protein	1.7		
lmo2255	Unknown	2.1		
lmo2263	Similar to unknown proteins	1.5		
lmo2269	Unknown	1.7		
lmo2311	Unknown	1.8	1.7	
lmo2391	Conserved hypothetical protein similar to <i>B. subtilis</i> YhfK protein	1.7		
lmo2432	Unknown	1.9	1.8	
lmo2454	Unknown	1.9	1.7	
lmo2574	Unknown		1.7	
lmo2670	Conserved hypothetical protein	1.6		
lmo2673	Conserved hypothetical protein	1.5		
lmo2723	Similar to unknown proteins		1.6	
lmo2724	Similar to unknown proteins	1.7		

^a Genes that met the criteria ($a \geq 1.5$ fold change and adjusted $P < 0.05$) for upregulation after exposure to PL, UV light, or UV

blocked PL.

^b Protein functions are based on annotations provided by ListiList (<http://genolist.pasteur.fr/ListiList/>), TIGR (<http://cmr.tigr.org/tigr-scripts/CMR/CmrHomePage.cgi>), and the KEGG Sequence Similarity Database (<http://www.genome.jp/kegg/ssdb/>).

^c Change(n-fold) indicates the transcriptional level ratio between *L. monocytogenes* 10403S cells exposed to light compared to untreated cells (e.g. $n = 2.0$ indicates a 2.0-fold higher transcript level after light exposure than untreated).

480 Table 3. Genes identified by microarray analysis to be down-regulated following exposure to PL, UV Light, or UV blocked PL^a

Protein category and gene	Protein function ^b	Change (n-fold) in cells exposed to ^c :		
		PL	UV light (254 nm)	UV blocked PL
(1)	(2)	(3)	(4)	(5)
Transcription or translation				
lmo2173	Similar to sigma-54-dependent transcriptional activator			-1.7
Cell membrane function				
lmo0186	Similar to <i>B. subtilis</i> YabE protein			-1.6
lmo0641	Similar to heavy metal-transporting ATPase			-1.5
lmo0683	Similar to chemotactic methyltransferase CheR			-2.0
lmo0689	Similar to CheA activity-modulating chemotaxis protein CheV			-1.6
lmo0843	Similar to <i>B. subtilis</i> protein YsdA			-1.6
lmo0912	Similar to transporters (formate)			-1.8
lmo1636	Similar to similar to ABC transporter (ATP-binding protein)			-2.9
lmo1634	Similar to Alcohol-acetaldehyde dehydrogenase			-17.5
lmo1637	Similar to membrane proteins			-3.3
lmo1699	Some similarities to methyl-accepting chemotaxis proteins			-1.5
lmo1852	Similar to putative mercuric ion binding proteins			-2.1
lmo1853	Similar to heavy metal-transporting ATPases			-2.0
lmo1884	Similar to xanthine permeases			-1.9
lmo2035 (<i>murG</i>)	Similar to peptidoglycan synthesis enzymes, putative phospho-N-acetylmuramoyl-pentapeptide-transferase			-2.0
lmo2036 (<i>murD</i>)	Similar to UDP-N-acetylmuramoylalanine D-glutamate ligase			-1.6
lmo2037 (<i>mraY</i>)	Similar to phospho-N-acetylmuramoyl-pentapeptide transferase			-1.7
lmo2504	Similar to cell wall binding proteins			-2.0
(1)	(2)	(3)	(4)	(5)

lmo2528 (<i>atpC</i>)	Highly similar to H ⁺ -transporting ATP synthase chain epsilon	-1.5
lmo2529 (<i>atpD</i>)	Highly similar to H ⁺ -transporting ATP synthase chain beta	-1.6
lmo2530 (<i>atpG</i>)	Highly similar to H ⁺ -transporting ATP synthase chain gamma	-1.7
lmo2531 (<i>atpA</i>)	Highly similar to H ⁺ -transporting ATP synthase chain alpha	-1.7
lmo2532 (<i>atpH</i>)	Highly similar to H ⁺ -transporting ATP synthase chain delta	-2.1
lmo2533 (<i>atpF</i>)	Highly similar to H ⁺ -transporting ATP synthase chain b	-1.7
lmo2534 (<i>atpE</i>)	Highly similar to H ⁺ -transporting ATP synthase chain c	-2.0
lmo2535 (<i>atpB</i>)	Highly similar to H ⁺ -transporting ATP synthase chain a	-1.6
lmo2536 (<i>atpI</i>)	Highly similar to ATP synthase subunit i	-1.8
lmo2634	Similar to <i>B. subtilis</i> YbaF protein	-1.6
lmo2715 (<i>cydD</i>)	Highly similar to ABC transporter (ATP-binding protein) required for expression of cytochrome BD	-1.7
lmo2716 (<i>cydC</i>)	Highly similar to ABC transporter required for expression of cytochrome BD	-1.7
lmo2717 (<i>cydB</i>)	Highly similar to cytochrome D ubiquinol oxidase subunit II	-1.5
lmo2530 (<i>atpG</i>)	Highly similar to H ⁺ -transporting ATP synthase chain gamma	-1.7
Motility		
lmo0681	Similar to flagellar biosynthesis protein FlhF	-2.1
lmo0682	Similar to flagellar hook-basal body protein FlgG	-2.2
lmo0685	Similar to motility protein (flagellar motor rotation) MotA	-2.6
lmo0686 (<i>motB</i>)	Similar to motility protein MotB	-2.2
lmo0696	Similar to flagellar hook assembly protein	-1.5
lmo0697	Similar to flagellar hook protein FlgE	-1.9
lmo0698	Weakly similar to flagellar switch protein	-2.6
lmo0699	Similar to flagellar switch protein FliM	-2.5
lmo0700	Similar to flagellar motor switch protein fliY	-2.1
lmo0705	Similar to flagellar hook-associated protein FlgK	-1.6
lmo0707	Similar to flagellar hook-associated protein 2 FliD	-2.0
(1)	(2)	(3) (4) (5)

lmo0710	Similar to flagellar basal-body rod protein flgB	-1.7
lmo0712	Similar to flagellar hook-basal body complex protein FliE	-1.7
lmo0713	Similar to flagellar basal-body M-ring protein fliF	-1.8
lmo0714	Similar to flagellar motor switch protein fliG	-2.0
lmo0716	Similar to H ⁺ -transporting ATP synthase alpha chain FliI, flagellar-specific	-2.0
Phosphotransferase systems		
lmo0096	Similar to PTS system mannose-specific, factor IIAB	-1.5
lmo0097	Similar to PTS system mannose-specific, factor IIC	-1.6
lmo0098	Similar to PTS system mannose-specific, factor IID	-1.5
lmo0875	Similar to PTS system, beta-glucoside enzyme IIB component	-1.5
lmo1972	Similar to pentitol PTS system enzyme II B component	-1.6
DNA/RNA metabolism		
lmo0280	Highly similar to anaerobic ribonucleotide reductase activator protein	-1.7
lmo1322 (nusA)	Highly similar to N utilization substance protein A (NusA protein)	-1.5
lmo1324	Conserved hypothetical protein, similar to B. subtilis YlxQ protein	-1.7
lmo1325 (<i>infB</i>)	Highly similar to translation initiation factor IF-2	-1.6
lmo1327 (<i>rbfA</i>)	Highly similar to ribosome-binding factor A	-1.8
lmo1596 (<i>rpsD</i>)	Ribosomal protein S4	-1.9
lmo1754 (<i>gatB</i>)	Glutamyl-tRNA(Gln) amidotransferase (subunit B)	-1.5
lmo1755 (<i>gatA</i>)	Glutamyl-tRNA(Gln) amidotransferase (subunit A)	-1.6
lmo1756 (<i>gatC</i>)	Glutamyl-tRNA(Gln) amidotransferase (subunit C)	-1.7
lmo2460	Similar to <i>B. subtilis</i> CggR hypothetical transcriptional regulator	-2.7
lmo2559 (<i>pyrG</i>)	Highly similar to CTP synthases	-1.9
lmo2611 (<i>adk</i>)	Highly similar to adenylate kinases	-1.8
lmo2612 (<i>secY</i>)	Highly similar to preprotein translocase subunit	-1.5
lmo2613 (<i>rplO</i>)	Ribosomal protein L15	-1.6
(1)	(2)	(3) (4) (5)

lmo2614 (<i>rpmD</i>)	Ribosomal protein L30	-1.9
lmo2615 (<i>rpsE</i>)	Ribosomal protein S5	-1.7
lmo2616 (<i>rplR</i>)	Ribosomal protein L18	-2.0
lmo2617 (<i>rplF</i>)	Ribosomal protein L6	-1.9
lmo2618 (<i>rpsH</i>)	Ribosomal protein S8	-1.9
lmo2619 (<i>rpsN</i>)	Ribosomal protein S14	-1.8
lmo2620 (<i>rplE</i>)	Ribosomal protein L5	-2.2
lmo2621 (<i>rplX</i>)	Ribosomal protein L24	-1.8
lmo2622 (<i>rplN</i>)	Ribosomal protein L14	-2.4
lmo2623 (<i>rpsQ</i>)	Ribosomal protein S17	-2.0
lmo2624 (<i>rpmC</i>)	Ribosomal protein L29	-1.8
lmo2625 (<i>rplP</i>)	Ribosomal protein L16	-1.7
lmo2626 (<i>rpsC</i>)	Ribosomal protein S3	-1.9
Metabolism		
lmo0355	Similar to Flavocytochrome C Fumarate Reductase chain A	-3.0
lmo0717	Similar to transglycosylase	-2.0
lmo0961	Similar to proteases	-1.7
lmo1091	Similar to glycosyltransferases	-1.6
lmo1151	Similar to <i>Salmonella typhimurium</i> PduA protein	-1.6
lmo1200	Similar to cobalamin biosynthesis J protein CbiJ	-1.6
lmo1672 (<i>menE</i>)	Similar to O-succinylbenzoic acid-CoA ligase	-1.6
lmo1803	Similar to FtsY of and SRP receptor alpha-subunit	-1.6
lmo1817	Weakly similar to thiamin <i>E. coli</i> pyrophosphokinase	-1.6
lmo1917 (<i>pflA</i>)	Similar to pyruvate formate-lyase	-1.6
lmo1989 (<i>leuC</i>)	Similar to 3-isopropylmalate dehydratase (large subunit)	-1.5
lmo2034 (<i>divIB</i>)	Similar to cell-division initiation protein divIB	-1.8
lmo2455 (<i>eno</i>)	Highly similar to enolase	-2.5
lmo2456 (<i>pgm</i>)	Highly similar to phosphoglycerate mutase	-3.1
(1)	(2)	(3) (4) (5)

lmo2457 (<i>tpi</i>)	Highly similar to triose phosphate isomerase	-3.3
lmo2458 (<i>pgk</i>)	Highly similar to phosphoglycerate kinase	-3.5
lmo2459 (<i>gap</i>)	Highly similar to glyceraldehyde 3-phosphate dehydrogenase	-1.8
lmo2635	Weakly similar to <i>E. coli</i> MenA protein	-1.9
lmo2636	Conserved hypothetical lipoprotein	-1.8
Other or hypothetical proteins		
lmo0047	Unknown	-1.7
lmo0350	Unknown	-1.5
lmo0573	Conserved hypothetical protein	-1.5
lmo0684	Unknown	-1.8
lmo0687	Unknown	-2.1
lmo0688	Similar to unknown protein	-2.0
lmo0701	Unknown	-2.9
lmo0702	Unknown	-2.9
lmo0703	Unknown	-1.6
lmo0704	Unknown	-1.6
lmo0715	Unknown	-2.0
lmo0718	Unknown	-1.8
lmo0731	Unknown	-1.6
lmo0788	Unknown	-2.7
lmo0954	Unknown	-2.2
lmo1257	Unknown	-3.5
lmo1326	Conserved hypothetical protein similar to <i>B. subtilis</i> YlxP protein	-1.7
lmo1700	Unknown	-1.7
lmo1796	Similar to unknown protein	-1.6
lmo1854	Similar to conserved hypothetical proteins	-2.9
lmo1966	Similar to unknown proteins	-1.7
lmo2029	Similar to unknown proteins	-1.5
(1)	(2)	(3) (4) (5)

lmo2116	Unknown	-1.6
lmo2151	Similar to unknown proteins	-1.5
lmo2258	Unknown	-1.8
lmo2410	Unknown	-1.8
lmo2486	Unknown	-3.2
lmo2487	Similar to <i>B. subtilis</i> YvIB protein	-2.2
lmo2567	Unknown	-2.7
lmo2568	Unknown	-3.3
lmo2594	Unknown	-1.6
lmo2669	unknown	-1.5
lmo2711	Similar to hypothetical proteins	-1.7

481

482 ^a Genes that met the criteria ($a \geq 1.5$ fold change and adjusted $P < 0.05$) for upregulation after exposure to PL, UV light, or UV

483 blocked PL.

484 ^b Protein functions are based on annotations provided by ListiList (<http://genolist.pasteur.fr/ListiList/>), TIGR (<http://cmr.tigr.org/tigr-scripts/CMR/CmrHomePage.cgi>), and the KEGG Sequence Similarity Database (<http://www.genome.jp/kegg/ssdb/>).

486 ^c Change(n-fold) indicates the transcriptional level ratio between *L. monocytogenes* 10403S cells exposed to light compared to
487 untreated cells (e.g. $n = 2.0$ indicates a 2.0-fold higher transcript level after light exposure than untreated).

488