zam-aem/zam02219/zam9444d19z | xppws | S=5 | 9/6/19 | 8:47 | ArtID: 01462-19 | DOI:10.1128/AEM.01462-19 | CE: CNV-se

Editor: Christopher A. Elkins | Section: Food Microbiology | Designation: T

AMERICAN SOCIETY FOR MICROBIOLOGY | Applied and Environmental Microbiology®

FOOD MICROBIOLOGY

Exposure to Broad-Spectrum Visible Light Causes Major Transcriptomic Changes in *Listeria monocytogenes* EGDe

AQ: au

Kristin Sæbø Pettersen,[a,b] Arvind Y. M. Sundaram,[c] Taran Skjerdal,[a] Yngvild Wasteson,[b] Anne Kijewski,[b] Toril Lindbäck,[b] Marina Aspholm[b]

[a]Norwegian Veterinary Institute, Oslo, Norway
[b]Norwegian University of Life Sciences, Oslo, Norway
[c]Norwegian Sequencing Centre, Department of Medical Genetics, Oslo University Hospital, Norway

ABSTRACT *Listeria monocytogenes*, the causative agent of the serious foodborne disease listeriosis, can rapidly adapt to a wide-range of environmental stresses, including visible light. This study shows that exposure of the *L. monocytogenes* EGDe strain to low-intensity, broad-spectrum visible light inhibited bacterial growth and caused altered multicellular behavior during growth on semisolid agar compared to when the bacteria were grown in complete darkness. These light-dependent changes were observed regardless of the presence of the blue light receptor (Lmo0799) and the stressosome regulator sigma B (SigB), which have been suggested to be important for the ability of *L. monocytogenes* to respond to blue light. A genome-wide transcriptional analysis revealed that exposure of *L. monocytogenes* EGDe to broad-spectrum visible light caused altered expression of 2,409 genes belonging to 18 metabolic pathways compared to bacteria grown in darkness. The light-dependent differentially expressed genes are involved in functions such as glycan metabolism, cell wall synthesis, chemotaxis, flagellar synthesis, and resistance to oxidative stress. Exposure to light conferred reduced bacterial motility in semisolid agar, which correlates well with the light-dependent reduction in transcript levels of flagellar and chemotaxis genes. Similar light-induced reduction in growth and motility was also observed in two different *L. monocytogenes* food isolates, suggesting that these responses are typical for *L. monocytogenes*. Together, the results show that even relatively small doses of broad-spectrum visible light causes genome-wide transcriptional changes, reduced growth, and motility in *L. monocytogenes*.

IMPORTANCE Despite major efforts to control *L. monocytogenes*, this pathogen remains a major problem for the food industry, where it poses a continuous risk of food contamination. The ability of *L. monocytogenes* to sense and adapt to different stressors in the environments enables it to persist in many different niches, including food production facilities and in food products. The present study shows that exposure of *L. monocytogenes* to low-intensity broad-spectrum visible light reduces its growth and motility and alters its multicellular behavior. Light exposure also caused genome-wide changes in transcript levels, affecting multiple metabolic pathways, which are likely to influence the bacterial physiology and lifestyle. In practical terms, the data presented in this study suggest that broad-spectrum visible light is an important environmental variable to consider as a strategy to improve food safety by reducing *L. monocytogenes* contamination in food production environments.

KEYWORDS EGDe, *Listeria monocytogenes*, broad-spectrum visible light, environmental stress, flagellar motility, growth inhibition, transcriptome

Citation Pettersen KS, Sundaram AYM, Skjerdal T, Wasteson Y, Kijewski A, Lindbäck T, Aspholm M. 2019. Exposure to broad-spectrum visible light causes major transcriptomic changes in *Listeria monocytogenes* EGDe. Appl Environ Microbiol 85:e01462-19. https://doi.org/10.1128/AEM.01462-19.

Editor Christopher A. Elkins, Centers for Disease Control and Prevention

Address correspondence to Marina Aspholm, marina.aspholm@nmbu.no.

Received 29 June 2019
Accepted 9 August 2019
Accepted manuscript posted online 6 September 2019
Published

zam-aem/zam02219/zam9444d19z | xppws | S=5 | 9/6/19 | 8:47 | ArtID: 01462-19 | DOI:10.1128/AEM.01462-19 | CE: CNV-se

Listeria monocytogenes is a foodborne opportunistic pathogen that causes the invasive disease listeriosis. Immunosuppressed individuals, the elderly, and pregnant women and their fetuses are at particularly high risk for acquiring invasive listeriosis (1). Listeriosis is characterized by bacteremia, meningoencephalitis, abortion, and neonatal sepsis (2). The overall fatality rate is between 20 and 30% of reported cases (1). *L. monocytogenes* exhibits tolerance to a wide range of environmental stressors, which facilitate its survival and replication in an extensive range of habitats, including soil, plant material, foods, and food-processing environments and in the intestinal tracts of mammals (3–5). In order to develop innovative strategies to control this pathogen, it is crucial to understand how it responds and adapts to stressors encountered in different environments.

The damaging effect of UV light on bacteria is well documented and is often applied for reducing bacterial contamination (6). It has been shown that the blue part of the visible-light spectrum (400 to 500 nm) has a bactericidal effect (7–13). The bactericidal effect of visible light is suggested to be related to endogenous production of reactive oxygen species, which have a dose-dependent and multitarget oxidizing effect on cellular components (7–11). Several studies have been conducted to explore the bactericidal effect of blue light for decontamination purposes, especially in combination with other stressors such as low temperature, salt, acidic pH, or light sensitizers (8, 9, 14–19).

Many environmental nonphototrophic bacteria, including *L. monocytogenes*, can sense and modulate their gene expression profiles and phenotypes in response to visible light of different wavelengths (20). It has been suggested that visible light can provide an early warning that facilitates bacterial adaptation to upcoming osmotic stress caused by water evaporation under sunlight (21). There are also nonphototropic bacteria that regulate their surface attachment and biofilm formation according to the presence of visible light (20). Some pathogenic bacteria, such as *Brucella abortus* and *Acinetobacter baumannii*, use light to assess their environment and to regulate their transition between environmental and host-associated life styles (20, 22, 23).

In *L. monocytogenes*, exposure to blue and red light induces transcription of the gene encoding the stressosome regulator sigma B (SigB) and consequently of genes belonging to the SigB regulon (SigB) (10, 21). *L. monocytogenes* senses blue light via the blue light photoreceptor (Lmo0799), whose activation triggers the transcription of *sigB* (21). Blue light is suggested to inhibit flagellar motility and increase invasiveness for Caco-2 enterocytes in a SigB- and blue light receptor-dependent manner (21). Furthermore, growth under oscillating light and dark conditions has been shown to cause an alternating translucent (not light exposed) and opaque (light exposed) ring pattern in *L. monocytogenes* colonies, indicating a blue-light-dependent and reversible multicellular behavior (11). The ring formation was dependent on both SigB and the blue light receptor, the positive regulatory factor A (PrfA) and the actin assembly-inducing protein (ActA) (11). *Bacillus subtilis*, which belongs to the same order as *L. monocytogenes* (i.e., the *Bacillales* order), contains a receptor which detects red light and has a broader spectral sensitivity than the blue light receptor (24). An orthologue to the *B. subtilis* red light receptor has not been identified in *L. monocytogenes*, and the mechanism behind the detection of red light is still unknown (21). The effect of red and blue light has been studied separately in *L. monocytogenes* and, based on mutational studies, it has been suggested that there is some cross talk or interference between red and blue light-triggered regulatory mechanisms (21). However, there is still very limited knowledge on the integrated response of *L. monocytogenes* to the full light spectrum and on how it regulates this bacterium's life style. In general, many questions regarding the physiological effects of visible light on nonphototrophic bacteria remains unanswered, and the use of "omics" technologies has been suggested as a tool to fill this knowledge gap (25).

The present study explored the effect of broad-spectrum visible light on *L. monocytogenes* by comparing the growth and transcriptomes of light-exposed cultures to those of cultures exposed to darkness. To capture the impact of temperature and

zam-aem/zam02219/zam9444d19z	xppws	S=5	9/6/19	8:47	ArtID: 01462-19	DOI:10.1128/AEM.01462-19	CE: CNV-se

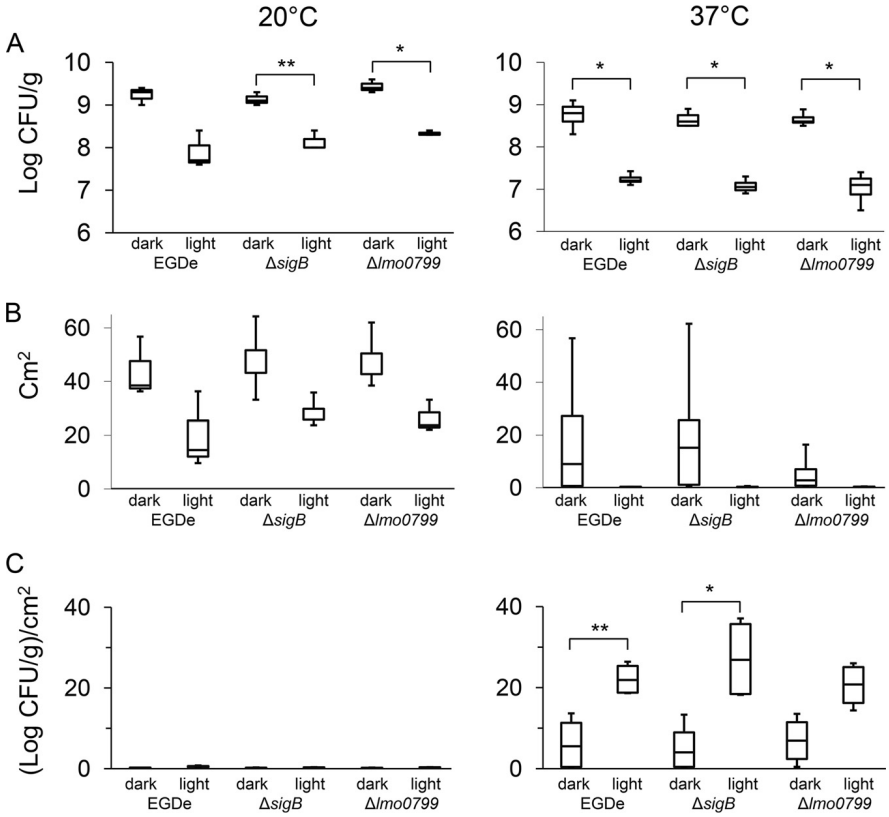

FIG 1 Growth of EGDe, EGDe Δ*sigB*, and EGDe Δ*lmo0799* strains, as indicated by log CFU/g (A), cm^2 (B), and bacterial density (C) after exposure to broad spectrum visible light at 20°C (N = 3) or 37°C (N = 4) for 12 and 7 days, respectively. In each box, the central mark indicates the median, and the bottom and top edges indicate the 25th and 75th percentiles, followed by the lower and upper adjacent values. Asterisks represent statistical differences from pairwise comparisons determined using two-tailed paired Student *t* tests (*, $P < 0.01$; **, $P < 0.05$).

exposure time on the transcriptomic response to light, the bacteria were exposed to visible light at 20°C for 20 and 180 min and at 37°C for 20 min. Functional analyses of selected significantly light-regulated pathways were performed to understand how altered gene regulation results in phenotypic differences between light- and dark-exposed samples. Together, the data presented here demonstrate that low-intensity, broad-spectrum visible light confers genome-wide changes in transcript levels and influences the growth and behavior of *L. monocytogenes*.

RESULTS AND DISCUSSION

Exposure to broad-spectrum visible light inhibits growth and causes altered multicellular behavior. To investigate the effect of low-intensity broad-spectrum visible light on the *L. monocytogenes* EGDe strain, it was grown for 7 days on semisolid agar plates under exposure of light or under complete darkness at 20 or 37°C. Exposure to light resulted in a reduced concentration of bacteria on the agar plates (log CFU/g agar) compared to cultures exposed to complete darkness at both temperature conditions tested, indicating that light exposure reduce bacterial growth (Fig. 1A). The colony areas were significantly reduced during light exposure at both 20 and 37°C (Fig. 1B), and a light-dependent increase in bacterial growth density was observed at 37°C (Fig. 1C). The differences in growth areas and growth densities observed between light- and dark-exposed cultures suggest that light exposure may alter the aggregative behavior of *L. monocytogenes*. The average dose of irradiance that each cell in a colony is exposed to will decrease when the cell density increases. The increased growth density may thus function as a protective strategy against light-induced stress. This assumption is supported by a previous study that shows that the inhibitory effect of

zam-aem/zam02219/zam9444d19z | xppws | S=5 | 9/6/19 | 8:47 | ArtID: 01462-19 | DOI:10.1128/AEM.01462-19 | CE: CNV-se

TABLE 1 Number of affected genes after light exposure at 20°C for 20 and 180 min and at 37°C for 20 min

Temp (°C)	Time (min)	No. of affected genes[a]					
		<0.5 log_2-fold change		0.5–2 log_2-fold change		>2 log_2-fold change	
		+	–	+	–	+	–
20	20	185	173	471	645	141	123
20	180	196	280	424	412	7	18
37	20	172	274	334	471	127	36

[a]The numbers of genes are categorized according to the log_2-fold change and extent of regulation. +, significantly upregulated genes; –, significantly downregulated genes.

blue light (470 nm) on *L. monocytogenes* was dependent on the cell density, since only cultures containing $<10^7$ CFU/ml were found to be growth inhibited by blue light exposure (10). Similarly, broad-spectrum, visible-light-induced growth inhibition was also observed when the EGDe strain and two different *L. monocytogenes* food isolates were cultured in liquid media at 20 and 37°C (Table S1). The reduced growth of light-exposed cultures compared to cultures grown in darkness were more pronounced at earlier time points of growth, when the bacterial density in the cultures were lower, and decreased when the cultures reached higher densities.

Exposure to visible light alters *L. monocytogenes* gene expression profiles. To get a genome-wide view on how broad-spectrum visible light influences various biological activities in *L. monocytogenes*, a transcriptomic analysis was performed on the EGDe strain grown in liquid media under exposure to broad-spectrum visible light or complete darkness at 20°C for 20 min, 20°C for 180 min, or 37°C for 20 min. The transcriptomes of the light-exposed cultures were compared to cultures exposed to complete darkness under the same time-temperature conditions. The exposure times at 20°C were selected to investigate the immediate response (20 min) and longer-term (180 min) adaptation to light, and individual genes were defined as significantly differentially expressed based on a $\geq log_2$-fold difference between light- and dark-exposed samples and an adjusted P value of <0.05.

Exposure to broad-spectrum visible light at 20°C for 20 min changed the transcript levels of 1,735 genes, 54% of which were downregulated, and 46% were upregulated (Table 1 and see Table S2 in the supplemental material). After 180 min of light exposure, the numbers of regulated genes were reduced to 1,336 (53% downregulated and 47% upregulated) and the number, of ≥2 log 2-fold up- and downregulated genes were also lower relative to cultures exposed to light for only 20 min (Table 1 and Table S2). Thus, the shift from darkness to light seemed to trigger a stronger and more complex transcriptional response compared to longer time exposures to light. However, a relatively large part of the regulated genes (n = 1,006) were shared between samples exposed to light for 20 and 180 min at 20°C, suggesting that they may represent a core set of genes regulated during light exposure at this temperature. Exposure to broad-spectrum visible light for 20 min at 37°C caused altered regulation of 1,414 genes, 55% of which were downregulated, and 45% were upregulated (Table 1 and Table S2). A total number of 913 differentially expressed genes were shared between light treatment at 20 and 37°C for 20 min. In all, 624 differentially expressed genes were shared across all time-temperature conditions (Fig. 2).

Light-dependent differently regulated metabolic pathways. KEGG pathway function analysis revealed that the differentially expressed genes (light versus darkness) participate in 18 metabolic pathways (Table 2). Altogether, six pathways (fructose mannose metabolism, pyrimidine metabolism, two-component system, bacterial chemotaxis, flagellar assembly, and the phosphotransferase system) were differentially regulated during light exposure across all time-temperature conditions tested. However, there were also differences between the conditions tested, which indicate that the response to light is both time and temperature dependent. During exposure to light for

zam-aem/zam02219/zam9444d19z | xppws | S=5 | 9/6/19 | 8:47 | ArtID: 01462-19 | DOI:10.1128/AEM.01462-19 | CE: CNV-se

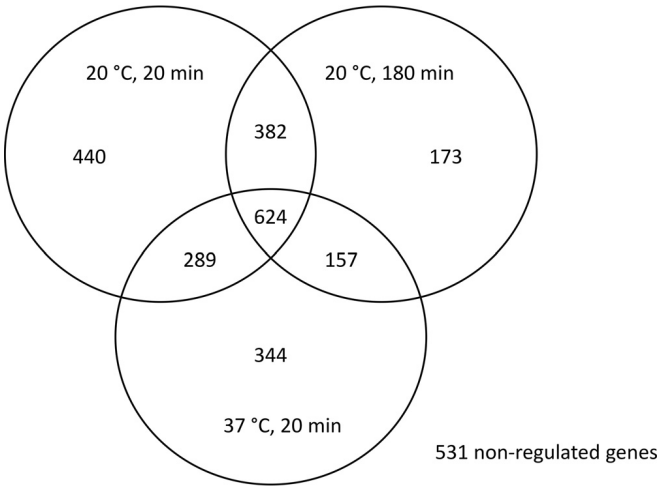

FIG 2 Venn diagram showing the number of differentially expressed genes across temperatures and time points and the overlap between the sets of differentially expressed genes.

20 min at 20°C, eight metabolic pathways were differently regulated. Seven of these pathways were also differently regulated after 180 min of light exposure at 20°C (Table 2 and Table S2). Furthermore, three pathways (pentose phosphate, microbial metabolism in diverse environments, and carbon metabolism) were only differentially regulated at the later time point. For several of the pathways that were differently regulated at both 20 and 180 min of light exposure at 20°C, a greater number of genes were affected at the earlier time point.

Light exposure at 37°C for 20 min resulted in altered regulation of genes belonging to 14 KEGG pathways, and 8 of these were also differentially regulated during light exposure at 20°C (Table 2 and Table S2). Six metabolic pathways, including the fatty acid biosynthesis, purine metabolism, peptidoglycan biosynthesis, aminoacyl-tRNA biosynthesis, amino sugar and nucleotide sugar metabolism, and the fatty acid metabolism, were only differently regulated during light exposure at 37°C. On the other hand, there were four pathways (pentose phosphate pathway, porphyrin metabolism, microbial metabolism in diverse environments, and carbon metabolism) that were only

TABLE 2 KEGG pathways affected by light exposure and the number of genes being differentially expressed

KEGG pathway	No. of genes	No. of affected genes[a]		
		20°C, 20 min	20°C, 180 min	37°C, 20 min
lmo00030 pentose phosphate pathway	35	NA	16	NA
lmo00051 fructose and mannose metabolism	56	38	20	29
lmo00061 fatty acid biosynthesis	14	NA	NA	10
lmo00230 purine metabolism	54	NA	NA	38
lmo00240 pyrimidine metabolism	41	29	17	18
lmo00500 starch and sucrose metabolism	56	31	NA	21
lmo00520 amino sugar and nucleotide sugar metabolism	34	NA	NA	21
lmo00550 peptidoglycan biosynthesis	19	NA	NA	15
lmo00860 porphyrin and chlorophyll metabolism	35	32	23	NA
lmo00970 aminoacyl-tRNA biosynthesis	92	NA	NA	19
lmo01120 microbial metabolism in diverse environments	150	NA	70	NA
lmo01200 carbon metabolism	84	NA	32	NA
lmo01212 fatty acid metabolism	15	NA	NA	10
lmo02020 two-component system	47	31	30	30
lmo02030 bacterial chemotaxis	12	10	11	12
lmo02040 flagellar assembly	26	23	24	26
lmo02060 phosphotransferase system	85	60	22	38
lmo03010 ribosome	75	NA	14	48

[a]NA, not affected (i.e., the pathway was not significantly affected).

zam-aem/zam02219/zam9444d19z | xppws | S=5 | 9/6/19 | 8:47 | ArtID: 01462-19 | DOI:10.1128/AEM.01462-19 | CE: CNV-se

differentially regulated during light exposure at 20°C (Table 2). A selection of the light-dependent differentially regulated pathways are further discussed below.

Porphyrins and flavins. A high number of the genes that were significantly downregulated during light exposure at 20°C are involved in porphyrin and flavin metabolism. A large proportion of these genes demonstrated a $>$2.0-$\log_2$-fold downregulation after 20 min of light exposure. Both porphyrins and flavins function as endogenous photosensitizers in microbial cells. They contain large conjugated systems that absorb energy from visible light and transition it to an excited electronic state (26). The excited porphyrin molecules may react directly with biological structures (type I reactions) or with molecular oxygen, generating excited singlet oxygen (type II reactions), which may react with and cause damage to bacterial proteins or to the DNA (26). It could therefore be a beneficial strategy for the bacterial cell to lower the level of endogenous photosensitizers to reduce damage from oxidative stress. The porphyrin pathway ends up in reducing the cobalamin (vitamin B_{12}) coenzyme. Notably, the transcriptome analyses showed that multiple genes involved in vitamin B_{12} synthesis were significantly downregulated during light exposure at 20°C, particularly after 20 min. It has long been known that vitamin B_{12} functions as a cofactor for many enzymes that catalyze a range of important biochemical reactions (27). Recently, it was discovered that vitamin B_{12} also controls gene expression in response to light by binding covalently to transcription factors (27, 28). The use of B_{12} as a light sensor is suggested to be a feature with a deep evolutionary history as proteins with B_{12} binding domains are widespread among species belonging to the *Bacteria*, *Archaea*, and *Eukarya* kingdoms. Vitamin B_{12} has also been shown to influence gene expression in *L. monocytogenes* by binding to RNA-based riboswitches (29). Light-dependent downregulation of genes involved in vitamin B_{12} synthesis could therefore have an indirect effect on the expression of multiple genes in *L. monocytogenes*.

To investigate whether extracellular vitamin B_{12} increases the sensitivity of *L. monocytogenes* to broad-spectrum visible light, we cultured the EGDe strain in light and in darkness in the presence vitamin B_{12}. Measurements of the optical density at 600 nm (OD_{600}) of the bacterial cultures did not show any significant differences in growth between bacteria grown in the presence of light with or without vitamin B_{12} (see Table S1 in the supplemental material). This may be due to absence of vitamin B_{12} uptake into the cell or indicate that other vitamin B_{12} derivatives are involved in light sensitivity. However, further studies are needed to understand the biological function of the light-dependent downregulation of vitamin B_{12} metabolism.

Protection against oxidative stress. Reactive oxygen species causes damage to carbohydrates, lipids, and proteins, and bacteria have evolved several different strategies to protect themselves against oxidative stress (30). The genes encoding superoxide dismutase (Lmo1439) and catalase (Lmo2785), which are both important for protection against oxidative stress, were upregulated during exposure to broad-spectrum visible light at all temperature conditions tested (Table 3). The pentose phosphate pathway, which produces NADPH, an important component in the defense mechanism against oxidative stress (31), was only upregulated after 180 min of light exposure at 20°C, which suggests that this protective mechanism comes into play after a longer time of light exposure.

Flagellar motility and chemotaxis. One of the most profound results from the transcriptomic analyses was the light-dependent downregulation of the flagellar assembly and chemotaxis genes (Fig. 3). Previous studies have shown that genes linked to flagellar motility in *L. monocytogenes* are expressed at temperatures between 20 and 25°C and transcriptionally downregulated at 37°C, although strain-dependent differences may occur (32–35). In the present study, exposure to broad-spectrum visible light resulted in downregulation of flagellar assembly genes at both 20 and 37°C. At 20°C, 23 of 26 (the total number of genes in the KEGG flagellar assembly pathway) flagellar assembly genes were downregulated after 20 min of light exposure. After 180 min of light exposure, 24 of the 26 flagellar assembly genes were downregulated. During light

zam-aem/zam02219/zam9444d19z	xppws	S=5	9/6/19	8:47	ArtID: 01462-19	DOI:10.1128/AEM.01462-19	CE: CNV-se

TABLE 3 Regulation of virulence determinants and genes of special interest (log_2-fold change)

Gene	Log_2-fold change			Gene description
	20°C, 20 min	20°C, 180 min	37°C, 20 min	
prfA	2.1	0.7	−0.5	Transcriptional regulator
sigB	2.1	0.9		RNA polymerase sigma factor
inlA	2.3	1.9	1.3	Internalin A
inlB	2.6	1.3	1.3	Internalin B
hly		0.4	−0.4	Listeriolysin O precursor
actA	−1.0			Actin-assembly inducing protein precursor
lmo0799	0.4	0.4	0.3	Blue light receptor
lmo1439	1.7	0.9	1.0	Superoxide dismutase
lmo2785	0.9	0.6	0.5	Catalase
argB	−1.8	−1.0	−0.8	Histidine kinase regulation
argC	−1.9	−1.1		
argD	−1.2	−0.8	−0.7	
argA	−0.9	−0.7	−0.5	
mouR	1.0		0.5	Transcriptional regulator
mogR	0.3	−0.4	0.3	Transcriptional regulator

exposure at 37°C for 20 min, all 26 genes were downregulated (less than a −1.0-log_2-fold change). The downregulation of flagellar genes at 37°C ranged from −3.1- to −1.4-log_2-fold changes, while the downregulation at 20°C was more variable, ranging from −4.4- to −0.5-log_2-fold changes (Table S2). To analyze the relative transcription level, compared to the normalization level across all samples, all light-temperature categories were gathered for comparison. In general, the flagellar assembly genes were most transcribed in the dark-exposed samples at 20°C, followed by light-exposed samples at 20°C (see Fig. S1 in the supplemental material). Consistent with what has been reported earlier, there was a much lower expression of flagellar genes at 37°C compared to at 20°C.

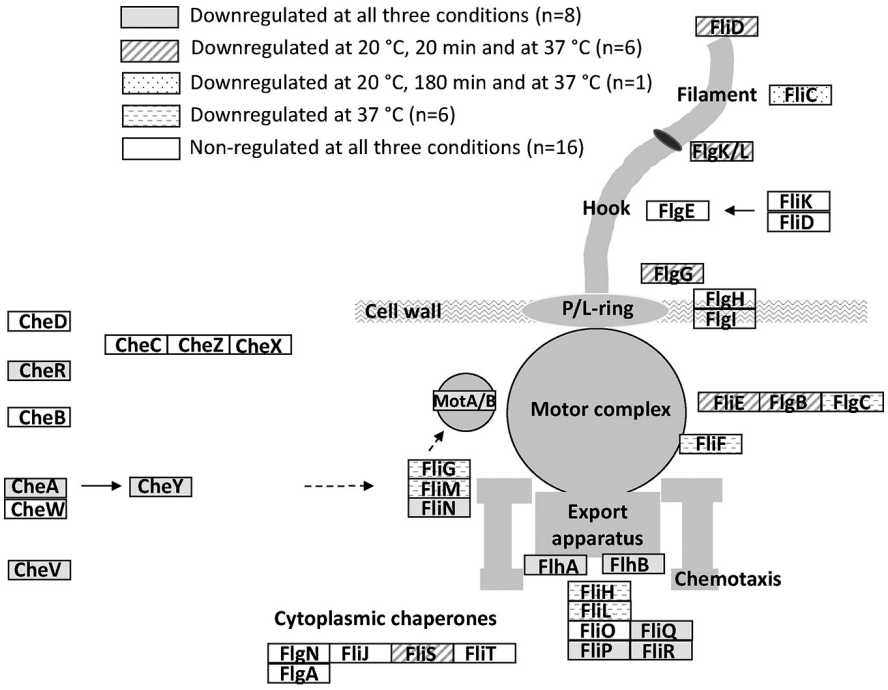

FIG 3 Downregulated genes (log_2-fold change of less than −1.0) in the chemotaxis and flagellar assembly pathways after exposure to broad-spectrum visible light. Different patterns of shading indicate the condition(s) the under which the downregulations were observed, according to the key. White indicates nonregulated.

zam-aem/zam02219/zam9444d19z	xppws	S=5	9/6/19	8:47	ArtID: 01462-19	DOI:10.1128/AEM.01462-19	CE: CNV-se

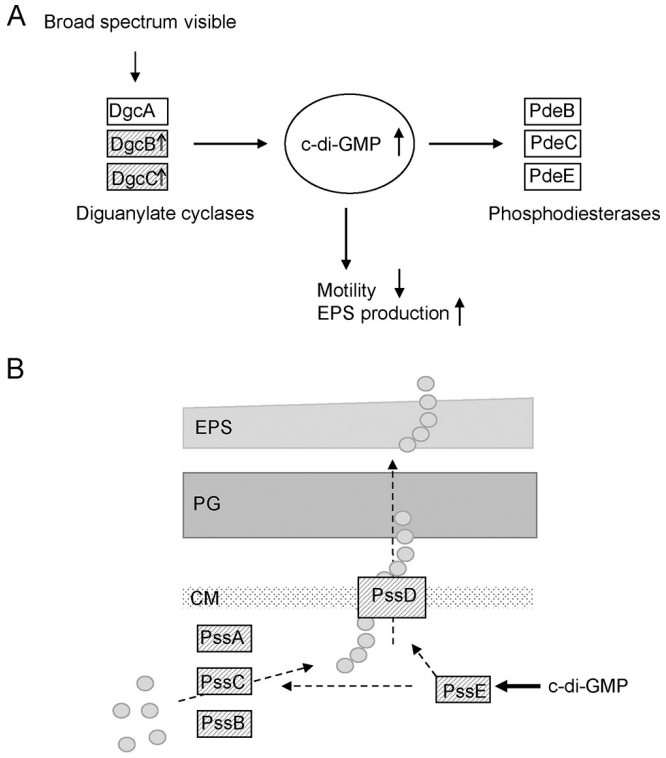

FIG 4 (A) Visible light causes upregulation of *dgcB* and *dgcC*, which likely will increase the intracellular concentration of c-di-GMP. (B) c-di-GMP positively regulates EPS production through PssE. Shaded boxes indicate genes encoding the proteins that are upregulated after exposure to light at 20°C. (The figure was adapted from reference 39 with permission from Oxford University.)

Two different food isolates (59792 and 59782) and the EGDe strain were tested for motility in soft agar, and all three strains showed the characteristic umbrella shaped motility when incubated in darkness at 20°C (Fig. S2). In contrast, the motility was severely reduced when the soft agar cultures were exposed to light at 20°C (Fig. S2). At 37°C, no motility could be detected in either light- or dark-exposed cultures (Fig. 2S). Together, these results indicate that visible light is an important factor that regulates flagellar motility in *L. monocytogenes* (21, 36, 37).

MogR is a transcriptional regulator that regulates swimming motility of *L. monocytogenes* in a temperature-dependent manner by repressing flagellar and chemotaxis genes (33, 38). In this study, only a slight upregulation of *mogR* was observed after 20 min of light exposure at both 20 and 37°C. After 180 min of light exposure at 20°C, it was −0.4 $\log_2$-fold downregulated (Table 3). This indicates that MogR is less important for light-dependent regulation of flagellar motility.

Light-induced expression of two diguanylate cyclases responsible for the synthesis of c-di-GMP. Numerous studies indicate that the second messenger 3′-5′ cyclic diguanylate monophosphate (c-di-GMP) negatively regulates flagellar motility and promotes bacterial adherence and biofilm formation (39). In *L. monocytogenes* it has previously been shown that c-di-GMP induces the expression of an exopolysaccharide that inhibits bacterial migration on semisolid agar, increases cellular aggregation, and confers enhanced tolerance to disinfectants and desiccation (40). Cyclic di-GMP is synthesized from two GTP molecules in a reaction catalyzed by diguanylate cyclase (Fig. 4A), and *L. monocytogenes* contains three genes encoding enzymes with diguanylate cyclase activity: *dgcA* (*lmo1911*), *dgcB* (*lmo1912*), and *dgcC* (*lmo2174*). In this study, exposure to broad-spectrum visible light triggered the upregulation of *dgcC* at all three time-temperature conditions tested (20°C and 20 min, a $\log_2$-fold change of 1.9; 20°C and 180 min, a $\log_2$-fold change of 1.1; and at 37°C and 20 min, a $\log_2$-fold change of 1.3), while *dgcB* was only upregulated during light exposure at 20°C for

zam-aem/zam02219/zam9444d19z | xppws | S=5 | 9/6/19 | 8:47 | ArtID: 01462-19 | DOI:10.1128/AEM.01462-19 | CE: CNV-se

20 min (a $\log_2$-fold change of 1.0). At 37°C, *dgcB* was weakly downregulated (a $\log_2$-fold change of −0.3). In contrast to the other two diguanylate cyclase genes in *L. monocytogenes*, *dgcA* was not differentially regulated during exposure to light at any of the time-temperature conditions tested.

Recent studies indicate that light exposure causes increased cell wall thickness in *L. monocytogenes*. Although not experimentally confirmed, this phenomenon was explained by increased production of extracellular polymeric substances (EPS) (10, 11). The *pssA-E* operon (*lmo0527-lmo0531*) has been linked to c-di-GMP-induced EPS biosynthesis in *L. monocytogenes* (40, 41). In the present study, all *pssA-E* genes were upregulated during light exposure at 20°C for 20 min and 180 min (between 1.7 and 1.9 $\log_2$-fold and between 0.5 and 0.7 $\log_2$-fold, respectively) (Fig. 4B, Table S1). To explore the effect of visible light on EPS production *L. monocytogenes* was cultured on agar plates containing Congo red either in the absence or presence of visible light. Dark- and light-exposed bacteria were also tested in a whole-cell Congo red depletion assay. No EPS production could be detected when the bacteria were grown at 20°C either in the presence of light or in darkness, even though transcriptional data indicated an increase in transcription of all *pssA-E* genes after light exposure at this temperature. At 37°C both methods detected EPS production, but there was no detectable difference in EPS production between light- and dark-exposed samples (data not shown). The latter result is in compliance with the transcriptional data which suggest that light exposure at 37°C do not affect transcription of the *pssA-E* genes.

***SigB* and the blue light receptor.** Upregulation of *sigB* was observed after exposure of the EGDe strain to broad-spectrum visible light for 20 and 180 min at 20°C ($\log_2$-fold changes of 2.1 and 0.9, respectively). However, this was not observed after light exposure at 37°C. The blue light receptor gene, Δ*lm0799*, was slightly upregulated (between 0.3 to 0.4 and 0.3 $\log_2$-fold change) in response to light exposure at all three time-temperature conditions tested (Table 3). The Δ*sigB* strain and the Δ*lm0799* strain, which lack SigB and the blue light receptor, respectively, did not demonstrate any observable differences in growth behavior on soft agar plates compared to the EGDe background strain (Fig. 1A). This is in contrast to what was reported by O'Donoghue et al., who observed that *L. monocytogenes* mutant strains lacking either SigB or the blue light receptor were less growth inhibited by blue light (460 to 470 nm, 1.5 to 2 mW cm^2) compared to the wild-type background strain (10). The different responses observed in the present study and in the study by O'Donoghue et al. (10) are likely due to differences in doses and wavelengths of the light the bacteria are exposed to. Similar to the results from the present study, NicAogáin et al. did not observe any significant difference in survival between the blue light receptor mutant (the strain used in the present study) and the wild-type EGDe background strain, when exposed to blue light (470 nm) in seawater (42). However, these researchers found that exposure to blue light reduced the survival of the Δ*sigB* mutant and suggested that the activity of SigB was regulated by other pathways than via the blue light receptor (42).

Virulence determinants and genes of special interest. In *L. monocytogenes*, the key virulence genes are expected to be most highly expressed at 37°C and almost silent at 30°C and is under the control of the temperature-sensing transcriptional regulator PrfA (43). Due to the complex regulatory network, it is difficult to predict the biological consequences of the broad-spectrum, visible-light-dependent upregulation of *prfA* transcription observed at 20°C. Only a slight upregulation of *prfA* expression was observed during light exposure at 37°C (Table 3). PrfA contributes to transcription of the membrane proteins Internalin A and Internalin B, which are involved in attachment and invasion of host cells, and they have also been suggested to mediate the adherence of *L. monocytogenes* to abiotic surfaces (44–46). In the present study, the genes encoding Internalin A and Internalin B (*inlA* and *inlB*, respectively) were upregulated under all light-temperature conditions tested (Table 3). This is consistent with results from a previous study, which showed that *inlA* and *inlB* were upregulated during exposure of *L. monocytogenes* to blue light (21). Since the *inl* genes were transcribed

zam-aem/zam02219/zam9444d19z | xppws | S=5 | 9/6/19 | 8:47 | ArtID: 01462-19 | DOI:10.1128/AEM.01462-19 | CE: CNV-se

during light exposure at both 20 and 37°C, there could be other factors, in addition to PrfA, that are involved in the regulation of *inlA* and *inlB*. Further studies are required to elucidate whether light influences attachment of *L. monocytogenes* to abiotic and biotic surfaces.

It has been suggested that that the actin-assembly inducing protein (ActA) is essential for coordinated light- and dark-dependent colony differentiation in *L. monocytogenes* (11). In the present study, the gene encoding the ActA precursor was downregulated after light exposure at 20°C for 20 min, but not after 180 min of light exposure, indicating that the downregulation may be an immediate short-term response to light-induced stress.

Conclusion and further perspectives. To interpret the functional elements involved in the response of *L. monocytogenes* to broad-spectrum visible light, the EGDe strain was exposed to light of wavelengths between 390 and 780 nm, with a maximum irradiance within the blue-green wavelengths of the spectrum. The simulated daylight cannot directly be compared to natural daylight since natural light contains a wider range of wavelengths and constantly changes the proportion of the different wavelengths during time of the day and with the season (47). If only comparing total radiant exposure (J/cm^2) used in the present study, with the sunlight, it corresponds roughly to 8- and 60-s exposures for 20 and 180 min, respectively. However, the energy will not give full information about the effect, since the wavelength spectrum differs between sunlight and the current light source used (e.g., UV), and the comparison has been made with the assumption that sunlight results in 0.1 W/cm^2.

The data presented here shows that exposure of *L. monocytogenes* to low intensities of broad-spectrum visible light reduces bacterial growth and induces major reprogramming of the bacterial transcriptome. Since light exposure at 20 and 37°C affects a different set of physiological pathways, the effect of light may be temperature dependent. The observed light-induced changes in transcription levels suggest that broad-spectrum visible light has a major influence on many biological processes in the bacterial cell and is an important environmental factor to consider both in research on this bacterium and for controlling it in food production environments. The use of low-intensity visible light as a mechanism to reduce growth of *L. monocytogenes* could have advantages compared to the use of high-intensity light (UV-light) for controlling this pathogen in food production facilities. For example, unlike UV-light, visible light passes through plastic, glass, and clear liquids. While rooms have to be evacuated when using high-intensity UV-light, one can still use rooms exposed low-intensity visible light. Visible light can therefore potentially be used as a method for continuous low-level control of *L. monocytogenes*.

L. monocytogenes has the ability to colonize abiotic surfaces, which contributes to its persistence in food processing facilities and increases the risk for contamination of food products (48). Several studies have addressed the potential for applying the toxic effect of visible light for disinfection in the food industry, both for surface decontamination and in food, by coating food products with photosensitizing compounds (14, 16, 18). Knowledge of the response and adaptation of *L. monocytogenes* to different intensities and wavelengths of visible light is therefore highly relevant for the further development and innovation of these strategies. The present study has highlighted genes and metabolic pathways that are differentially expressed during exposure of *L. monocytogenes* to broad-spectrum visible light and provides a valuable basis for more-detailed functional analyses to understand how light affects the bacterial behavior. There is also need for further understanding on how light of different wavelengths and intensities affect *L. monocytogenes*.

MATERIALS AND METHODS

Bacterial strains and growth conditions on agar plates. All of the strains used in this study are listed in Table 4. The EGDe strain and its Δ*sigB* and Δ*lmo0799* derivative strains were kindly provided by Jörgen Johansson at Umeå University, Umeå, Sweden (10, 11). The EGDe strain was chosen since its whole-genome sequence is available and well annotated (49).

zam-aem/zam02219/zam9444d19z | xppws | S=5 | 9/6/19 | 8:47 | ArtID: 01462-19 | DOI:10.1128/AEM.01462-19 | CE: CNV-se

TABLE 4 *L. monocytogenes* strains used in the present study

Strain	Reference	Isolate source	Serogroup
EGDe	49	Rabbit	IIa (I/2a)
EGDe Δ*sigB*	10	Laboratory strain	
EGDe Δ*lmo0799*	10	Laboratory strain	
59789	58	RTE chicken	IIa
59792	58	Meatballs	IIc

The bacteria were grown in brain heart infusion broth (BHI; catalog no. 237500, Bacto; Becton Dickinson, Sparks, MD) or on semisolid BHI agar (0.3%) and exposed to broad-spectrum visible light or kept in darkness at 20 or 37°C. Prior to growth on semisolid agar, bacteria from freeze stocks were grown on blood agar plates for 24 h at 37°C, transferred to BHI broth, and incubated overnight at 37°C. The OD_{600} of the overnight cultures was measured, and 2-μl portions were then transferred from the cultures to the center of 0.3% semisolid BHI agar plates. The plates were sealed with parafilm and incubated at 20 or 37°C for 12 and 7 days, respectively, under continuous light exposure or in darkness. On the last day of incubation, the diameters of the growth zones were registered. To determine the bacterial concentration on the agar plates, the colonies and the agar from the culture plates were transferred to stomacher bags, weighted, diluted 1:2 (wt/wt) with BHI, and plated on blood agar in proper dilutions for enumeration. Linear regression analysis of the log CFU/g, including the presence of light, the effect of temperature, and the agar weight, resulted in an adjusted R^2 of 90.5, meaning that the model can explain 90.5% of the variation in log CFU/g observed. However, only temperature and the presence of light were significant ($P > t = 0.000$). The effect of weight was not significant ($P > t = 0.629$), and when excluding this predictor from the model, the adjusted R^2 value slightly improved, resulting in 90.7. Therefore, the effect of the agar weight was assumed to be limited. All experiments were performed in triplicates or quadruplets.

To explore the effect of broad-spectrum visible light on growth in liquid medium, 4-ml portions of BHI cultures were grown in small cell-culture bottles (catalog no. 83.3911.002; Sarstedt AG & Co., Germany) at 20 or 37°C. The bottles were placed 17 to 20 cm from the light source. Growth was assessed by measuring the OD_{600}. To not expose "dark" cultures to light, a new parallel culture was used for each measurement both for dark- and light-exposed cultures. Dark-exposed culture bottles were wrapped in two layers of aluminum foil.

Preparation of RNA for sequencing. For the global transcriptomic analysis, *L. monocytogenes* EGDe strain was grown on blood agar plates for 24 h at 37°C. Seven single colonies, representing seven biological replicates, were transferred into seven separate tubes containing BHI broth, followed by incubation at 20 or 37°C until an OD_{600} of 0.8 was reached. The cultures were diluted 10,000 times in BHI broth before 4-ml portions of the cultures were transferred to six × seven cell culture bottles (Sarstedt AG). All six bottles were wrapped up in aluminum foil and incubated at 20 or 37°C. After reaching an OD_{600} of 0.6 to 0.8, half of the bottles were exposed to light by removal of the aluminum foil. The control samples were incubated next to the light-exposed samples. Immediately after light/dark exposures, three bottles incubated under the same conditions were pooled, RNAprotect bacterial reagent (catalog no. 76506; Qiagen) was added according to the manufacturer's instructions, and the samples were stored at −80°C until RNA isolation. Three bottles were used per biological replicate to get enough cells without increasing the volume of the culture in one bottle. RNA isolation was performed by using an RNeasy minikit (Qiagen), including enzymatic lysis (lysozyme [50 μl of 100 mg/ml] and mutanolysin [Sigma-Aldrich, catalog no. M9901-5KU; 10 μl of 50 U]), proteinase K digestion (Qiagen, catalog no. 19131; 15 μl of 25 mg/ml) and mechanical disruption (bead beating, 6 m/s for 40 s [1×] using a Precellys lysing kit; Bertin Technologies, catalog no. 03961-1-005). DNase treatment was performed in columns (RNase-free DNase set; Qiagen, catalog no. 79254) according to the manufacturer's instructions. The quality and the quantity of the total RNA samples were estimated by using an Agilent Bioanalyzer (Agilent Technologies) and NanoDrop One (Thermo Scientific), respectively. RIN values ranged from 8.8 to 9.9, the 260/280 ratio ranged from 1.8 to 2.1, and the 260/230 ratio ranged from 1.3 to 2.4; all samples were immediately depleted of rRNA by using Ribo-Zero magnetic kit bacteria (Illumina, catalog no. MRZB12424). The quality and quantity of the RNAs were measured after depletion using Bioanalyzer Pico Chips and a NanoDrop apparatus.

Light conditions. A Juwel Aquarium Discover Life light source (Day 24W T5; 9,000 K) was used to expose *L. monocytogenes* cultures to broad-spectrum visible light. The distance between the light source and the samples was held at 16 to 18 cm. Measurement of the blue light was done by using a Solar Light Co. PMA2100 detector (Blue Light Safety, serial number 2894; Solar Light Co., Philadelphia, PA), and the irradiation level was measured to 0.0002259 W/cm² × 60 s/min × *x* min = 0.014 J/cm² per min. The total radiant exposures (in J/cm²) were 0.8 and 6.1 for 20 and 180 min, respectively. The blue light radiant exposures (in J/cm²) were 0.3 and 2.4 for 20 and 180 min, respectively.

RNA sequencing. The transcriptomic analysis was performed on the EGDe strain grown under dark conditions in liquid media before exposure to light or darkness at 20°C for 20 min, at 20°C for 180 min, or at 37°C for 20 min. Two different exposure times were selected at 20°C to investigate the immediate (early) response to light exposure (20 min) and longer-term (180 min) adaptation to light. The longer exposure time was limited to 180 min to ensure RNA was harvested in the exponential growth phase. The light-exposed samples were only compared to corresponding samples kept in complete darkness (controls). Individual genes were defined as significantly differentially expressed based on a $\log_2$-fold

difference between light- and dark-exposed samples and an adjusted *P* value of <0.05. Altogether, 42 libraries (7 biological replicates for each treatment) were prepared and sequenced on an Illumina HiSeq AQ: K 3000 apparatus (150-bp PE reads) using a TruSeq stranded total RNA kit (Illumina) at the Norwegian Sequencing Centre, Oslo, Norway. The raw sequence reads were submitted to the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA506549. Bbduk v34.56 (50) was used for cleaning the raw data before alignment with Hisat2 v2.0.5 (51) using genome and annotation references from the ensemble bacteria (genome [Listeria_monocytogenes_egd_e.ASM19603v1.dna.toplevel.fa.gz from ENSEMBL BACTERIA] and annotation [Listeria_monocytogenes_egd_e.ASM19603v1.38.gtf.gz ENSEMBL BACTERIA]). Counting aligned reads was done with FeatureCounts v1.4.6-p1 (52), and finally DESeq2 v1.18.1 (53) was used for analysis of differentially regulated genes.

The experimental design included seven biological replicates from each condition. However, one control sample (dark) at 20°C for 20 min, two samples exposed to light at 20°C for 20 min, one sample exposed to light at 20°C for 180 min, and one sample exposed to light at 37°C for 20 min were removed due to low percentages of alignment (10.6 to 37.4% feature count alignment). In addition, due to outliers AQ: L defined by the cluster dendrogram and principal component analysis (Fig. S3), two more samples were removed (one control sample [dark] at 20°C for 20 min and one control sample [dark] at 37°C for 20 min). In all, five replicates were analyzed from both dark- and light-exposed cultures at 20 min and 20°C, six replicates were analyzed from both light conditions at 20 min and 37°C, and six and seven replicates were analyzed from 180 min and 20°C light- and dark-exposed conditions, respectively. Downstream analysis was performed according to the samples shown by the PCA analysis given in Fig. S1, which had a feature count alignment ranging from 71 to 83.4%. Output files from Deseq2 were first sorted on adjusted *P* values below 0.05. Conversion to the Entrez gene ID was performed using DAVID Bioinformatics Resources 6.8 (NIAID/NIH https://david.ncifcrf.gov/tools.jsp [54]). After conversion, Pathview was used for functional pathway analysis and visualization (https://pathview.uncc.edu/ [55–57]).

Congo red assays. For visualization of EPS production, *L. monocytogenes* was grown on plates containing 20 g/liter TSB (Difco), 6.1 g/liter peptone (Difco), 3.5% agar (Oxoid), and 25 μg/ml Congo red. After incubation for 48 h, the colors of the colonies were compared by visual inspection. To detect differences in EPS production in liquid cultures, *L. monocytogenes* was cultured in a media containing 20 g/liter TSB and 6.1 g/liter peptone for 48 h in light or in darkness. After incubation, the cells were washed once in phosphate-buffered saline and adjusted to OD_{600} of 0.5, and Congo red was added to the cultures to a final concentration of 20 μg/ml, followed by further incubation for 20 min at room temperature. The cells were then pelleted at 10,000 $\times$ *g* for 5 min, and the absorbance of the supernatant was measured at 500 nm to detect the depletion of Congo red from the media.

Motility test. An umbrella motility test was performed in glass tubes containing 5 ml of soft agar (20 g/liter TSB [Difco], 6.1 g/liter peptone [Difco], 3.5% agar [Oxoid]). Overnight cultures of *L. monocytogenes* strains were inoculated into the soft agar, and the tubes were incubated under light exposure (17 to 20 cm next to the light source) or in darkness (wrapped in aluminum foil) for 24 h at 37°C or for 48 h at 20°C.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AEM.01462-19.

SUPPLEMENTAL FILE 1, PDF file, 0.6 MB.

SUPPLEMENTAL FILE 2, XLSX file, 0.4 MB.

ACKNOWLEDGMENT

We thank Terje Christensen for sharing his competence in photobiology and for performing the light measurements.

This study is included in the project "Pathogens in the Food Chain," funded by the Norwegian Research Council (221663-F40).

K.S.P. and T.L. performed the experiments, with assistance from A.K. K.S.P. and A.Y.M.S. performed the bioinformatics analyses, the statistical analyses, and the initial interpretation. K.S.P. wrote the initial draft of the manuscript. All authors were involved AQ: M in the study design, final interpretation, and manuscript preparation.

REFERENCES

AQ: N

1. Hazards EPanel oB, Ricci A, Allende A, Bolton D, Chemaly M, Davies R, Fernández Escámez PS, Girones R, Herman L, Koutsoumanis K, Nørrung B, Robertson L, Ru G, Sanaa M, Simmons M, Skandamis P, Snary E, Speybroeck N, Ter Kuile B, Threlfall J, Wahlström H, Takkinen J, Wagner M, Arcella D, Da Silva Felicio MT, Georgiadis M, Messens W, Lindqvist R. 2018. *Listeria monocytogenes* contamination of ready-to-eat foods and the risk for human health in the EU. Efs2 16:e05134. https://doi.org/10.2903/j.efsa.2018.5134.
2. Ramaswamy V, Cresence VM, Rejitha JS, Lekshmi MU, Dharsana KS, Prasad SP, Vijila HM. 2007. Listeria: review of epidemiology and pathogenesis. J Microbiol Immunol Infect 40:4–13.
3. Weis J, Seeliger HP. 1975. Incidence of *Listeria monocytogenes* in nature. Appl Microbiol 30:29–32.
4. Sauders BD, Durak MZ, Fortes E, Windham K, Schukken Y, Lembo AJ, Jr, Akey B, Nightingale KK, Wiedmann M. 2006. Molecular characterization of *Listeria monocytogenes* from natural and urban environments. J Food Prot 69:93–105. https://doi.org/10.4315/0362-028X-69.1.93.
5. Sauders BD, Overdevest J, Fortes E, Windham K, Schukken Y, Lembo A,

zam-aem/zam02219/zam9444d19z	xppws	S=5	9/6/19	8:47	ArtID: 01462-19	DOI:10.1128/AEM.01462-19	CE: CNV-se

Wiedmann M. 2012. Diversity of *Listeria* species in urban and natural environments. Appl Environ Microbiol 78:4420–4433. https://doi.org/10.1128/AEM.00282-12.
6. Uesugi AR, Hsu LC, Worobo RW, Moraru CI. 2016. Gene expression analysis for *Listeria monocytogenes* following exposure to pulsed light and continuous ultraviolet light treatments. Food Sci Technol 68:579–588. https://doi.org/10.1016/j.lwt.2016.01.007.
7. Endarko E, Maclean M, Timoshkin IV, MacGregor SJ, Anderson JG. 2012. High-intensity 405 nm light inactivation of *Listeria monocytogenes*. Photochem Photobiol 88:1280–1286. https://doi.org/10.1111/j.1751-1097.2012.01173.x.
8. McKenzie K, Maclean M, Timoshkin IV, MacGregor SJ, Anderson JG. 2014. Enhanced inactivation of *Escherichia coli* and *Listeria monocytogenes* by exposure to 405 nm light under sublethal temperature, salt and acid stress conditions. Int J Food Microbiol 170:91–98. https://doi.org/10.1016/j.ijfoodmicro.2013.10.016.
9. Ghate VS, Ng KS, Zhou W, Yang H, Khoo GH, Yoon WB, Yuk HG. 2013. Antibacterial effect of light emitting diodes of visible wavelengths on selected foodborne pathogens at different illumination temperatures. Int J Food Microbiol 166:399–406. https://doi.org/10.1016/j.ijfoodmicro.2013.07.018.
10. O'Donoghue B, NicAogain K, Bennett C, Conneely A, Tiensuu T, Johansson J, O'Byrne C. 2016. Blue-light inhibition of *Listeria monocytogenes* growth is mediated by reactive oxygen species and is influenced by sigmaB and the blue-light sensor Lmo0799. Appl Environ Microbiol 82:4017–4402. https://doi.org/10.1128/AEM.00685-16.
11. Tiensuu T, Andersson C, Ryden P, Johansson J. 2013. Cycles of light and dark co-ordinate reversible colony differentiation in *Listeria monocytogenes*. Mol Microbiol 87:909–924. https://doi.org/10.1111/mmi.12140.
12. Murdoch LE, Maclean M, Endarko E, MacGregor SJ, Anderson JG. 2012. Bactericidal effects of 405 nm light exposure demonstrated by inactivation of *Escherichia*, *Salmonella*, *Shigella*, *Listeria*, and *Mycobacterium* species in liquid suspensions and on exposed surfaces. Sci World J 2012:137805–137805. https://doi.org/10.1100/2012/137805.
13. Ghate V, Leong AL, Kumar A, Bang WS, Zhou W, Yuk HG. 2015. Enhancing the antibacterial effect of 461 and 521 nm light emitting diodes on selected foodborne pathogens in Trypticase soy broth by acidic and alkaline pH conditions. Food Microbiol 48:49–57. https://doi.org/10.1016/j.fm.2014.10.014.
14. Maclean M, McKenzie K, Anderson JG, Gettinby G, MacGregor SJ. 2014. 405 nm light technology for the inactivation of pathogens and its potential role for environmental disinfection and infection control. J Hosp Infect 88:1–11. https://doi.org/10.1016/j.jhin.2014.06.004.
15. Brovko L. 2010. Photodynamic treatment: a new efficient alternative for surface sanitation. Adv Food Nutr Res 61:119–147. https://doi.org/10.1016/B978-0-12-374468-5.00003-9.
16. Tosati JV, de Oliveira EF, Oliveira JV, Nitin N, Monteiro AR. 2018. Light-activated antimicrobial activity of turmeric residue edible coatings against cross-contamination of *Listeria innocua* on sausages. Food Control 84:177–185. https://doi.org/10.1016/j.foodcont.2017.07.026.
17. Lin SL, Hu JM, Tang SS, Wu XY, Chen ZQ, Tang SZ. 2012. Photodynamic inactivation of methylene blue and tungsten-halogen lamp light against food pathogen *Listeria monocytogenes*. Photochem Photobiol 88:985–991. https://doi.org/10.1111/j.1751-1097.2012.01154.x.
18. Josewin SW, Ghate V, Kim M-J, Yuk H-G. 2018. Antibacterial effect of 460 nm light-emitting diode in combination with riboflavin against *Listeria monocytogenes* on smoked salmon. Food Control 84:354–361. https://doi.org/10.1016/j.foodcont.2017.08.017.
19. Hessling M, Spellerberg B, Hoenes K. 2017. Photoinactivation of bacteria by endogenous photosensitizers and exposure to visible light of different wavelengths: a review on existing data. FEMS Microbiol Lett 364:fnw270. https://doi.org/10.1093/femsle/fnw270.
20. Gomelsky M, Hoff WD. 2011. Light helps bacteria make important lifestyle decisions. Trends Microbiol 19:441–448. https://doi.org/10.1016/j.tim.2011.05.002.
21. Ondrusch N, Kreft J. 2011. Blue and red light modulates SigB-dependent gene transcription, swimming motility and invasiveness in *Listeria monocytogenes*. PLoS One 6:e16151. https://doi.org/10.1371/journal.pone.0016151.
22. Mussi MA, Gaddy JA, Cabruja M, Arivett BA, Viale AM, Rasia R, Actis LA. 2010. The opportunistic human pathogen *Acinetobacter baumannii* senses and responds to light. J Bacteriol 192:6336–6345. https://doi.org/10.1128/JB.00917-10.
23. Swartz TE, Tseng TS, Frederickson MA, Paris G, Comerci DJ, Rajashekara G, Kim JG, Mudgett MB, Splitter GA, Ugalde RA, Goldbaum FA, Briggs WR, Bogomolni RA. 2007. Blue-light-activated histidine kinases: two-component sensors in bacteria. Science 317:1090–1093. https://doi.org/10.1126/science.1144306.
24. Ávila-Pérez M, van der Steen JB, Kort R, Hellingwerf KJ. 2010. Red light activates the σ(B)-mediated general stress response of *Bacillus subtilis* via the energy branch of the upstream signaling cascade. J Bacteriol 192:755–762. https://doi.org/10.1128/JB.00826-09.
25. van der Horst MA, Key J, Hellingwerf KJ. 2007. Photosensing in chemotrophic, non-phototrophic bacteria: let there be light sensing too. Trends Microbiol 15:554–562. https://doi.org/10.1016/j.tim.2007.09.009.
26. Afonso SG, Enriquez de Salamanca R, Batlle AM. 1999. The photodynamic and non-photodynamic actions of porphyrins. Braz J Med Biol Res 32:255–266. https://doi.org/10.1590/s0100-879x1999000300002.
27. Cheng Z, Yamamoto H, Bauer CE. 2016. Cobalamin's (vitamin B_{12}) surprising function as a photoreceptor. Trends Biochem Sci 41:647–650. https://doi.org/10.1016/j.tibs.2016.05.002.
28. Padmanabhan S, Jost M, Drennan CL, Elias-Arnanz M. 2017. A new facet of vitamin B_{12}: gene regulation by cobalamin-based photoreceptors. Annu Rev Biochem 86:485–514. https://doi.org/10.1146/annurev-biochem-061516-044500.
29. Mellin JR, Tiensuu T, Becavin C, Gouin E, Johansson J, Cossart P. 2013. A riboswitch-regulated antisense RNA in *Listeria monocytogenes*. Proc Natl Acad Sci U S A 110:13132–13137. https://doi.org/10.1073/pnas.1304795110.
30. Van Acker H, Coenye T. 2017. The role of reactive oxygen species in antibiotic-mediated killing of bacteria. Trends Microbiol 25:456–466. https://doi.org/10.1016/j.tim.2016.12.008.
31. Spaans SK, Weusthuis RA, van der Oost J, Kengen S. 2015. NADPH-generating systems in bacteria and archaea. Front Microbiol 6:742–742. https://doi.org/10.3389/fmicb.2015.00742.
32. Dons L, Rasmussen OF, Olsen JE. 1992. Cloning and characterization of a gene encoding flagellin of *Listeria monocytogenes*. Mol Microbiol 6:2919–2929. https://doi.org/10.1111/j.1365-2958.1992.tb01751.x.
33. Grundling A, Burrack LS, Bouwer HG, Higgins DE. 2004. *Listeria monocytogenes* regulates flagellar motility gene expression through MogR, a transcriptional repressor required for virulence. Proc Natl Acad Sci U S A 101:12318–12323. https://doi.org/10.1073/pnas.0404924101.
34. Peel M, Donachie W, Shaw A. 1988. Temperature-dependent expression of flagella of *Listeria monocytogenes* studied by electron microscopy, SDS-PAGE, and Western blotting. J Gen Microbiol 134:2171–2178. https://doi.org/10.1099/00221287-134-8-2171.
35. Way SS, Thompson LJ, Lopes JE, Hajjar AM, Kollmann TR, Freitag NE, Wilson CB. 2004. Characterization of flagellin expression and its role in *Listeria monocytogenes* infection and immunity. Cell Microbiol 6:235–242. https://doi.org/10.1046/j.1462-5822.2004.00360.x.
36. Caly D, Takilt D, Lebret V, Tresse O. 2009. Sodium chloride affects *Listeria monocytogenes* adhesion to polystyrene and stainless steel by regulating flagella expression. Lett Appl Microbiol 49:751–756. https://doi.org/10.1111/j.1472-765X.2009.02735.x.
37. Tresse O, Lebret V, Benezech T, Faille C. 2006. Comparative evaluation of adhesion, surface properties, and surface protein composition of *Listeria monocytogenes* strains after cultivation at constant pH of 5 and 7. J Appl Microbiol 101:53–62. https://doi.org/10.1111/j.1365-2672.2006.02968.x.
38. Shen A, Higgins DE. 2006. The MogR transcriptional repressor regulates nonhierarchal expression of flagellar motility genes and virulence in *Listeria monocytogenes*. PLoS Pathog 2:e30. https://doi.org/10.1371/journal.ppat.0020030.
39. Purcell EB, Tamayo R. 2016. Cyclic diguanylate signaling in Gram-positive bacteria. FEMS Microbiol Rev 40(5):753–773. https://doi.org/10.1093/femsre/fuw013.
40. Chen L-H, Köseoğlu VK, Güvener ZT, Myers-Morales T, Reed JM, D'Orazio SEF, Miller KW, Gomelsky M. 2014. Cyclic di-GMP-dependent signaling pathways in the pathogenic firmicute *Listeria monocytogenes*. PLoS Pathog 10:e1004301. https://doi.org/10.1371/journal.ppat.1004301.
41. Köseoğlu VK, Heiss C, Azadi P, Topchiy E, Güvener ZT, Lehmann TE, Miller KW, Gomelsky M. 2015. *Listeria monocytogenes* exopolysaccharide: origin, structure, biosynthetic machinery and c-di-GMP-dependent regulation. Mol Microbiol 96:728–743. https://doi.org/10.1111/mmi.12966.
42. NicAogáin K, Magill D, O'Donoghue B, Conneely A, Bennett C, O'Byrne CP. 2018. Solar irradiance limits the long-term survival of *Listeria monocytogenes* in seawater. Lett Appl Microbiol 66:169–174. https://doi.org/10.1111/lam.12831.
43. Lebreton A, Cossart P. 2017. RNA- and protein-mediated control of

Listeria monocytogenes virulence gene expression. RNA Biol 14:460–470. https://doi.org/10.1080/15476286.2016.1189069.

44. Chen B-Y, Kim TJ, Jung Y-S, Silva J. 2008. Attachment strength of *Listeria monocytogenes* and its internalin-negative mutants. Food Biophys 3:329–332. https://doi.org/10.1007/s11483-008-9090-7.
45. Gaillard JL, Berche P, Frehel C, Gouin E, Cossart P. 1991. Entry of *Listeria monocytogenes* into cells is mediated by internalin, a repeat protein reminiscent of surface antigens from gram-positive cocci. Cell 65:1127–1141. https://doi.org/10.1016/0092-8674(91)90009-n.
46. Dramsi S, Biswas I, Maguin E, Braun L, Mastroeni P, Cossart P. 1995. Entry of *Listeria monocytogenes* into hepatocytes requires expression of InlB, a surface protein of the internalin multigene family. Mol Microbiol 16:251–261. https://doi.org/10.1111/j.1365-2958.1995.tb02297.x.
47. Thorington L. 1985. Spectral, irradiance, and temporal aspects of natural and artificial light. Ann N Y Acad Sci 453:28–54. https://doi.org/10.1111/j.1749-6632.1985.tb11796.x.
48. Carpentier B, Cerf O. 2011. Review: persistence of *Listeria monocytogenes* in food industry equipment and premises. Int J Food Microbiol 145:1–8. https://doi.org/10.1016/j.ijfoodmicro.2011.01.005.
49. Murray EGD, Webb RA, Swann M. 1926. A disease of rabbits characterized by a large mononuclear leucocytosis, caused by a hitherto undescribed bacillus *Bacterium monocytogenes* (n.sp.). J Pathol 29:407–439. https://doi.org/10.1002/path.1700290409.
50. Bushnell B. 2014. BBMap: a fast, accurate, splice-aware aligner. Lawrence Berkeley National Lab, Berkeley, CA.
51. Kim D, Langmead B, Salzberg SL. 2015. HISAT: a fast spliced aligner with low memory requirements. Nat Methods 12:357. https://doi.org/10.1038/nmeth.3317.
52. Liao Y, Smyth GK, Shi W. 2014. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. Bioinformatics 30:923–930. https://doi.org/10.1093/bioinformatics/btt656.
53. Love MI, Huber W, Anders S. 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol 15:550. https://doi.org/10.1186/s13059-014-0550-8.
54. Huang da W, Sherman BT, Lempicki RA. 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 4:44–57. https://doi.org/10.1038/nprot.2008.211.
55. Luo W, Brouwer C. 2013. Pathview: an R/Bioconductor package for pathway-based data integration and visualization. Bioinformatics 29:1830–1831. https://doi.org/10.1093/bioinformatics/btt285.
56. Luo W, Pant G, Bhavnasi YK, Blanchard JSG, Brouwer C. 2017. Pathview Web: user friendly pathway visualization and data integration. Nucleic Acids Res 45:W501–W508. https://doi.org/10.1093/nar/gkx372. AQ: P
57. Luo W, Friedman MS, Shedden K, Hankenson KD, Woolf PJ. 2009. GAGE: generally applicable gene set enrichment for pathway analysis. BMC Bioinformatics 10:161. https://doi.org/10.1186/1471-2105-10-161.
58. Skjerdal T, Reitehaug E, Cudjoe K, Næss T, Folgerø B, Framstad K. 2010. Listeria shelf life of ready-to-(h)eat red and white meat products, poster. Food Microbiology 2010, Copenhagen, Denmark.
59. Plavskii VY, Mikulich AV, Tretyakova AI, Leusenka IA, Plavskaya LG, Kazyuchits OA, Dobysh II, Krasnenkova TP. 2018. Porphyrins and flavins as endogenous acceptors of optical radiation of blue spectral region determining photoinactivation of microbial cells. J Photochem Photobiol B 183:172–183. https://doi.org/10.1016/j.jphotobiol.2018.04.021.
60. Leimeister-Wachter M, Domann E, Chakraborty T. 1992. The expression of virulence genes in *Listeria monocytogenes* is thermoregulated. J Bacteriol 174:947–952. https://doi.org/10.1128/jb.174.3.947-952.1992.
61. Johansson J, Mandin P, Renzoni A, Chiaruttini C, Springer M, Cossart P. 2002. An RNA thermosensor controls expression of virulence genes in *Listeria monocytogenes*. Cell 110:551–561. https://doi.org/10.1016/s0092-8674(02)00905-4.
62. Autret N, Raynaud C, Dubail I, Berche P, Charbit A. 2003. Identification of the agr locus of *Listeria monocytogenes*: role in bacterial virulence. Infect Immun 71:4463–4471. https://doi.org/10.1128/iai.71.8.4463-4471.2003.
63. Riedel CU, Monk IR, Casey PG, Waidmann MS, Gahan CG, Hill C. 2009. AgrD-dependent quorum sensing affects biofilm formation, invasion, virulence and global gene expression profiles in *Listeria monocytogenes*. Mol Microbiol 71:1177–1189. https://doi.org/10.1111/j.1365-2958.2008.06589.x.
64. Vivant A-L, Garmyn D, Gal L, Hartmann A, Piveteau P. 2015. Survival of *Listeria monocytogenes* in soil requires AgrA-mediated regulation. Appl Environ Microbiol 81:5073–5084. https://doi.org/10.1128/AEM.04134-14.
65. Pinheiro J, Lisboa J, Pombinho R, Carvalho F, Carreaux A, Brito C, Pöntinen A, Korkeala H, dos Santos NS, Morais-Cabral JH, Sousa S, Cabanes D. 2018. MouR controls the expression of the *Listeria monocytogenes* Agr system and mediates virulence. Nucleic Acids Res 46:9338–9352. https://doi.org/10.1093/nar/gky624.

JOBNAME: AUTHOR QUERIES PAGE: 1 SESS: 2 OUTPUT: Fri Sep 6 08:48:19 2019
/rich4/zam-aem/zam-aem/zam02219/zam9444d19z

AUTHOR QUERIES

AUTHOR PLEASE ANSWER ALL QUERIES 1

AQau—Please confirm the given-names and surnames are identified properly by the colors. ■= Given-Name, ■= Surname

AQA—Is it correct that the same term—"SigB"—represents both items?

AQB—Table 1 has been edited in accordance with ASM style. Please check it (and any additional tables) carefully. Check the table column headings (including the units, if any) and footnotes (if any) in particular.

AQC—In Table 2, should column 1 be separated into a "Gene" column ("lmo00030", etc.) and a "KEGG pathway" column ("Pentose phosphate pathway", etc.)? Also, should the "lmo" terms be italicized and/or identified as genes?

AQD—Is the sentence that begins with "This may" correctly edited?

AQE—Please clarify/revise/define the term "*pssA-E* operon". Consider the following excerpt from the *ASM Style Manual*: "An operon, a segment of genomic DNA containing a structural gene or a linear sequence of structural genes, may be designated, e.g., *ompAB*, *lacZYA*, *ompAB-fepD*, or even *ompA–D* **if defined as the *ompA-ompD* operon**. *Note that the letters indicating the loci for the same gene may or may not be close to each other in the alphabet*. If you see something like *ompA-D*, ask the author to define the designation (explain whether it represents a fusion of *ompA* and *ompD* or an operon comprising *ompA* and *ompD* and the genetic material between the two genes)." These operon terms appear only in this paragraph in this paper.

AQF—Do you mean "weighed"?

AQG—Is the meaning of "$P > t = 0.000$" clear? Did you mean "(temperature, $P > 0.000$)" or perhaps just "($P > 0.000$)"? See also the next sentence.

AQH—What does "six × seven" mean?

AQI—Is "6 m/s" OK here ("6 meters/second)"? Also, what does the "[1×]" mean? Can "once" be used instead?

AQJ—What is "RIN" here? Please define or explain this term if it will not be readily understood.

AQK—What is "PE" here?

AQL—Was the term "PCA" spelled out correctly here?

AQM—Should authors M.A., T.S., and/or Y.W. be mentioned specifically in this paragraph?

AQN—Please spell out and/or clarify "Hazards EPanel oB."

AUTHOR QUERIES

AUTHOR PLEASE ANSWER ALL QUERIES 2

AQO—Original reference 24 appeared to be a duplicate of original reference 10, so it has been deleted, and the remaining references have been renumbered. Likewise, original reference 67, a duplicate of original reference 40, has also been deleted. Original references 43, 46, 47, 51, 52, 53, and 54 were not cited in the text or tables, so they were moved to the end of your references (and are now numbered as references 59 to 65). References 59 to 65 must either be cited or tables in the text under these new numbers or else removed.

AQP—Your original reference 64 (which included only "Gage FH") was deleted. That reference was not cited in the text, and it appeared to be either the same as (or similar to) original reference 65 ("GAGE", etc.), now reference 57, or else it looked like it might be unpublished data (e.g., "F. H. Gage, unpublished data"), which would normally only be cited in the text. If this was an actual reference, however, it can be reinstated if a complete reference citation is provided.