CPS 2020 RFP
FINAL PROJECT REPORT

Project Title
Control of *Listeria monocytogenes* in processing/packing plants using antimicrobial blue light (aBL)

Project Period
January 1, 2021 – December 31, 2021 (extended to January 31, 2022)

Principal Investigator
Francisco Diez-Gonzalez, PhD
University of Georgia
Center for Food Safety
1109 Experiment Street
Griffin, GA 30223
T: 770-228-7284
E: fdiez@uga.edu

Co-Principal Investigator
Govindaraj Dev Kumar, PhD
University of Georgia
Center for Food Safety
Griffin, GA 30223
T: 770-467-6094
E: goraj@uga.edu

Objectives

1. *Determine optimal emission dose for decontamination of free cells and biofilms of Listeria monocytogenes on stainless steel, alone and in the presence of a photosensitizer.*
2. *Evaluate antimicrobial blue light (aBL) efficacy for Listeria reduction on processing-related surfaces.*
3. *Assess aBL decontamination effectiveness at a pilot-plant scale.*

Funding for this project provided by the Center for Produce Safety through:
TDA 2020 Specialty Crop Block Grant Program & CPS Campaign for Research

FINAL REPORT

Abstract

Surface disinfection is critical for controlling *Listeria monocytogenes* in food processing environments. Antimicrobial blue light (aBL) in the 400–470 nm wavelength range has been reported to kill a wide range of microbes. aBL is an emerging antimicrobial intervention technology, but research on treatment of biofilms on inert surfaces was largely nonexistent. The goal of this study was to assess the potential of aBL for mitigation of *L. monocytogenes* in fresh produce processing/packaging plants, focusing on surface decontamination.

A series of experiments were conducted, using five *L. monocytogenes* strains selected on their ability to form biofilms and their fresh produce origin. Culture mixtures of these strains were used as dried cells and biofilms on stainless steel coupons. The incorporation to surfaces of a photosensitizer, gallic acid (GA), was also evaluated as an aBL enhancing adjuvant. Dried cells also were applied to other material coupons that included high-density polyethylene (HDPE), polystyrene (PS), polyvinyl chloride (PVC), and rubber (RUB). Low (5 log CFU/cm^2) and high (7 log CFU/cm^2) inoculum levels were used. Light emitting diode (LED) lamps emitting at 405, 420, and 460 nm wavelengths were used for the surface exposure at different doses ranging from 20 to 4,000 J/cm^2. The durations of exposure ranged from 2 to 24 h, depending on the lamp intensity. Following exposure, standard microbiological analyses were conducted on the inoculated coupons and surfaces to measure the extent of viable count reduction. Statistical analyses were performed using ANOVA. Confocal laser scanning microscopy (CLSM) with Syto®9 and propidium iodide staining was used to observe the biofilm structures. The aBL effect was also determined after inoculating SS, HDPE, and brushes that simulate packing plant surfaces.

Treatments at 405 nm (2,672 J/cm^2,16 h) reduced the counts of dried cells inoculated onto SS coupons by 3.1 log CFU/cm^2. In most experiments where GA was used, no differences were detected with controls, and in some cases the counts of GA treated samples were greater. Two types of biofilms were tested—dry and wet—and each were grown with either full-strength or diluted (1:10) tryptic soy broth. Dry and wet biofilms treated at 405 nm (at 2,672 J/cm^2) had from 3.2 to 4.2 log CFU/cm^2 fewer viable cells, respectively, indicating that aBL was less bactericidal against dry biofilms. Biofilms had greater survival to lower doses than did dried cells, and biofilm micrographs displayed shifts in biofilm structure from live to dead cells after aBL treatment, confirming its vast effectiveness. The highest dose tested at 420 nm (967 J/cm^2) reduced viable counts on biofilms by 1.9 log CFU/cm^2 ($P<0.05$). At 460 nm, after exposure to 800 J/cm^2, biofilm counts were reduced from 6.9 to 5.3 log CFU/cm^2 ($P<0.05$).

At 405 nm, the effectiveness of aBL varied depending on the surface material, with the least reduction of *L. monocytogenes* on PVC, 2.5 log CFU/cm^2 after 2,672 J/cm^2 ($P<0.05$); on PS, cells were inactivated by 4.5 log CFU/cm^2 after 2,672 J/cm^2. Exposure to 420 and 460 nm also caused significant reductions. Treatments at 420 nm (1,440 J/cm^2) resulted in reductions of 3.2 log CFU/cm^2 on PVC and 3.8 CFU/cm^2 on RUB ($P<0.05$). At 460 nm after 800 J/cm^2 on SS, cell counts were reduced from 6 to 4.8 log CFU/cm^2 ($P<0.05$). Under pilot plant conditions, at 102 cm from the 405 nm lamp, more than 1.5 log CFU/cm^2 were inactivated after a 740 J/cm^2 dose on SS and HDPE surfaces.

This is the first study that observed inactivation of *L. monocytogenes* biofilms on inert surfaces with aBL. aBL could inactivate *L. monocytogenes* on different materials, but its effectiveness was influenced by wavelength and type of material. The aBL technology can be environmentally friendly, safe, and adaptable to different production systems. The use of aBL for mitigation of *L. monocytogenes* may enhance the microbial safety of diverse fresh produce products resulting in improved public health, consumer trust, satisfaction and a better market for producers and retailers.

Background

L. monocytogenes is one of the major concerns for environmental contamination of ready-to-eat foods due to its widespread distribution and its ability to survive well in the environment of packing and processing related surfaces and equipment (1). The 2014-15 outbreak linked to caramel apples stressed the need for additional interventions because the grower's packing process was the likely source of contamination, as *L. monocytogenes* was detected on the packing line and exposed porous surfaces were present (2). Similarly, the investigation of another outbreak linked to stone fruits identified the fruit packing facility as the contamination point (3). In dairy foods, listeriosis outbreaks continue to occur even in Hispanic cheeses that have been pasteurized, which suggests environmental contamination. The most recent case linked consumption of fresh Hispanic cheeses to 15 infections (4). These events justify the idea of developing novel alternatives for processing and packing environment treatments.

In recent years, the application of blue light has been explored and adapted for multiple purposes. In material sciences, ultraviolet (UV) and blue light (BL) lamps have been widely used for 3D printing for controlled photopolymerization of different synthetic materials (5). The use of blue light has also been adopted to enhance plant health and nutritional quality of crops and medicinal plants (6). A recent study at the University of Georgia tested blue light on blueberries to enhance food quality and shelf life (7). These multiple applications have led to a rapid development of flexible and low-cost light emitting diodes (LED) technologies.

The use of ultraviolet light (UV), in particular UV-C which has a wavelength shorter than 280 nm, has been used as a disinfectant for many years in clinical, biomedical, laboratory and food production applications (8). Its widespread utilization, however, is limited by its detrimental effects on eyes and skin. Because UV light damages DNA, it can have mutagenic effects on human cells. A novel method capable of complementing other available interventions is the application of antimicrobial blue light (aBL). The aBL consists of safe, visible blue light in the spectrum of 400-470 nm (9). **Figure 1** shows the aBL position within the light spectrum. Within this spectrum, the visible color changes gradually from purplish-blue to blue from lower to higher frequencies. Thus, a variety of terms such as violet-blue, violet light or high-intensity narrow-spectrum light have also been used to refer to aBL.

Multiple studies have reported the antimicrobial effectiveness of aBL. Hope et al. reported aBL to be active against *Provotellaceae* (10). In another report, the growth of *Salmonella* and methicillin-resistant *Staphylococcus aureus* (MRSA) was suppressed by aBL at a 470 nm wavelength (11). Similarly, Abana and colleagues observed that aBL at 455 nm inhibited *E. coli* growth (12). Another promising use for aBL was reported in aquaculture after successful inactivation of fish pathogens, including *Vibrio angillarum*, *Edwardsiella tarda* and *Streptococcus* (13). In 2016, Halstead et al. reported the successful application of aBL against a range of nosocomial wound pathogens, such as *Acinetobacter baumanii, Enterobacter coloacae, Pseudomonas aeruginosa, Enterococcus faecium* and *Klebsialla pneumonia*, in liquid cultures and biofilms (14).

Due to its safety and applicability, aBL has been tested for environmental decontamination of hospital rooms (15, 16) and is considered an emerging technology for clinical and hospital decontamination (17). The potential effect of aBL against *L. monocytogenes* has been reported by a few studies. The inactivation of *L. monocytogenes* by aBL in liquid and agar media was first reported in 2012 in two separate studies, achieving a reduction of 5 log CFU/ml with 185 J/cm^2 (18, 19). Kim and colleagues observed that *L. monocytogenes* viability was more susceptible to 405 nm aBL than were *Staphylococcus* and *Bacillus* strains under refrigeration conditions, and they concluded that the effect of aBL was due to damage to the cell membrane (20). Another study described the cellular mechanisms involved in *L. monocytogenes* inactivation by aBL (21). In the first study of aBL applied to control *L. monocytogenes* in fresh produce, counts on inoculated cantaloupes treated with 1,210 J/cm^2 at 405 nm were reduced by 3 log CFU/cm^2 (22).

These reports clearly indicate that *L. monocytogenes* is susceptible to aBL and that this technology could be applied to its environmental control.

Before this project there was no report on the application of aBL for mitigation of *L. monocytogenes* on surfaces intended for produce processing/packing plants. The goal of this project was to assess the application of aBL for control of *L. monocytogenes* in fresh produce processing/packaging plants, mainly focusing on surface decontamination for the first time.

Research Methods and Results

Experimental Methods

Bacterial strains and culture conditions: A 5-strain mixture of *L. monocytogenes*, which included strains ATCC 19115 (human isolate), ATCC 19117 (sheep isolate), "Coleslaw" (from coleslaw), G1091 (from coleslaw), and 2011L-2624 (from cantaloupe outbreak), was used in this study. The first three strains were selected because of their strong biofilm forming ability. Each strain was activated from stock cultures by three successive 24 h transfers in 25 ml tryptic soy broth (TSB) supplemented with 0.6% yeast extract (TSBYE) and incubated at 35°C for 24 h. After the final incubation each of the 5 strains were streaked individually on tryptic soy agar (TSA) with 0.6% yeast extract (TSAYE) and placed in an incubator at 35°C for 48 h. The bacterial cells were scraped off the agar plates using a sterile inoculation loop and re-suspended into sterile phosphate buffered saline (PBS). The suspensions were diluted to attain a final concentration of 6 or 8 log CFU/ml and were pooled to form cocktails. The cocktails were used to evaluate aBL antimicrobial activities.

Target materials and target material inoculation: Stainless steel (SS), high density polyethylene (HDPE), polystyrene (PS), polyvinyl chloride (PVC), and rubber (RUB) 25 mm × 75 mm coupons were used for this study to represent the constitutive materials of food contact surfaces, totes/bins, tarps, containers, pipes, and flooring. Target surfaces were inoculated with 5-7 log CFU per coupon for dry cells and used to grow biofilms (below). Once inoculated with cells, the surfaces were dried for 2 h in a biosafety cabinet. For Objective 3, trays and a packing plant machine were used as SS surfaces in addition to flat HDPE plates and segments of roller brushes. Brushes were inoculated by dipping the bristles into a cell suspension containing approx. 7 log CFU/ml for 1 h and then drying overnight in a biosafety cabinet.

Biofilm growth: SS coupons were submerged into strain mixture cultures and incubated at 37°C for 48 h. Coupons were rinsed with PBS to eliminate planktonic cells and used for aBL treatment directly or after drying. Two nutrient levels were used for biofilm growth, either regular TSA or 1:10 diluted TSA. For confocal observations the mixture cultures were added to 8-well chamber slides (Nunc™ II; Lab-Tek™; Fisher Scientific) and then left to form biofilms at 37°C for 48 h under aerobic conditions. For CSLM, the chambers were rinsed with NaCl (8.5 g/L) and refilled with NaCl containing 5 µM Syto®9 (1:1000 dilution from a Syto®9 stock solution of the LIVE/DEAD BacLight™ viability kit, Molecular Probes, LifeTechnologies, Eugene, OR).

Blue light treatments: Inoculated coupons or wells with dried cells or biofilms at an initial count of approximately 6 log CFU per plate were allowed to dry for 48 h or treated within 2 h after removal (wet biofilms). For Objectives 1 and 2, commercially available LED arrays at 405, 420 and 460 nm were tested. The lamp descriptions are: 405 nm blue light LED array lamp (200 W, Fastobuy), 405 nm blue light LED array lamp (60 W, Kikappo, Inc.), 405 nm blue light LED array lamp (100 W, Kikappo, Inc.), 420 nm LED array lamp (3 W, Xinhongye), and 460 nm LED array lamps (25 W, Byingo). Lamps were placed from 10 to 20 cm of separation from inoculated

coupons and light exposure was conducted for up to 24 h. A radiometer to measure light intensity and a Gassen spectrometer to measure wavelength were also used. Emission doses as J/cm^2 were calculated by measuring the light intensity with the radiometer and multiplying the W/cm^2 values by the time in seconds. For Objective 3, high intensity 405 and 460 nm lamps with a maximal irradiation emission of 2,200 and 3,300 W/cm^2, respectively (Honle, Panacol-USA, Torrington, CT) were used to treat surfaces inoculated with dried cells. Three treatment conditions were used: 1) short distance (9 cm) and multiple short exposure cycles (ON: 40 s, OFF: 3-5 min, to keep the temperature below 50°C) for a total of 5 h; 2) mid distance (25 cm), surface temperature controlled with underneath cooling packs for 2 h; and 3) long separation (102 cm) and continuous exposure for 18 h.

Microbiological analysis: Treated coupons were placed in plastic bags containing PBS and transferred to an ultrasonic bath for 2 min sonication to recover cells. The contents were transferred into tubes and vortexed, and the viable cells released from coupons in the PBS suspension were serially diluted and spread in triplicate onto TSA plates. Petri plates were incubated for 24 h and colonies were counted, and CFU/surface plate were calculated. For Objective 3, inoculated treated surfaces were swabbed twice and the cotton tips were sonicated inside plastic bags containing PBS, which were processed as described above.

Evaluation of biofilm disruption: Biofilm structure changes were visualized using CSLM with a Zeiss LSM confocal laser scanning microscope (Carl Zeiss Microscopy). Serial images were captured and processed by Zeiss Zen 2.3 software (Carl Zeiss) following staining with cell fluorescent markers: SYTO®9/propidium iodide, and LIVE/DEAD BacLight™ viability kit.

Statistical analysis and data collection: Mean bacterial counts after each treatment were recorded as log CFU/cm^2. All experiments were performed with at least two independent trials in triplicate. Significant differences among bacterial populations and biofilm density were determined using ANOVA (analysis of variance) using JMP Pro 14 (SAS Institute, Cary, NC). A centralized server was used for recording data as soon as it was generated. Experimental raw data was collected and entered by the laboratory researchers. After the completion of each experiment replicate, the PI was notified about the data online and shared it with the co-PI. Any discrepancy with previous replicate or possible experimental error were highlighted through additional communication, and this was a topic of discussion for the next team meeting. The post-doc was responsible for the calculation of averages and dispersion measures, graphic representation of data, and statistical analysis of differences. Similarly, any secondary data entry was recorded and made readily available for the PI and co-PI. Additional data analysis and graphical presentation was conducted by the PI and co-PI and shared with the team.

Results

Objective 1

The specific aims were to 1) assess the aBL antimicrobial effect at three wavelengths at several doses; 2) determine the influence of application of a photosensitizer (gallic acid, GA); 3) compare the effect on dried cells and biofilms; and 4) characterize the aBL effect on biofilm viability and structure.

Treatments at 405 nm (2,672 J/cm^2, 16 h) reduced the counts of dried cells inoculated onto SS coupons by 3.1 log CFU/cm^2. Application of GA resulted in an additional reduction of 1 log CFU/cm^2 only at 668 J/cm^2. In most other experiments where GA was used, no differences were detected with controls, and in some cases the counts of GA treated samples were greater. We tested the same emission doses/times against biofilms as well. Two types of biofilms were

tested—dry and wet—and each were grown with either full-strength or diluted (1:10) tryptic soy broth. Dry and wet biofilms treated at 405 nm (2,672 J/cm^2 dose) had from 3.2 to 4.2 log CFU/cm^2 fewer viable cells, respectively, indicating that aBL was less bactericidal against dry biofilms (**Fig. 1**). Based on this observation only dry surface biofilms were tested for the rest of the project as a target for inactivation. The experiment with and without GA confirmed the effectiveness of aBL at 405 nm against biofilms, where the 2,672 J/cm^2 dose again caused more than 3 log CFU/cm^2 reduction (**Fig. 2A**). However, biofilms had greater survival to lower doses than did dried cells, including after application of the the photosensitizer (Ps), i.e., gallic acid (**Fig. 2B**). Furthermore, biofilm micrographs displayed shifts in biofilm structure from live to dead cells after aBL treatment, confirming its vast effectiveness (**Fig. 3**). At 460 nm, viable counts were reduced from 6 to 4.8 log CFU/cm^2 after exposure to 800 J/cm^2 (**Fig. 4A**), and almost the same effect was observed against biofilms, where the final biofilm reduction reached 1.7 log CFU/cm^2 (**Fig. 4B**). Similar experiments were completed at 420 nm, and the data is currently undergoing analysis. However, based on results analyzed thus far, the dose obtained after 16 h exposures delivered from different wavelengths seem to achieve significant reductions of *Listeria*'s viability.

Objective 2

The specific aim was to determine the influence of material composition on the reduction of viable counts of cells dried on coupons.

At 405 nm, the effectiveness of aBL varied depending on the surface material, with the least reduction of dried cell viability on PVC (from 2 log CFU/cm^2 after 668 J/cm^2 to 4 log CFU/cm^2 after 4,008 J/cm^2); dried cells on PS were reduced by 4 log CFU/cm^2 after 668 J/cm^2 doses (**Fig. 5A**). Reductions were also observed after exposing *L. monocytogenes* to 420 and 460 nm, however, with smaller reductions noted. Treatments at 420 nm (240 J/cm^2, 4 h or 1,440 J/cm^2, 24 h) resulted in reductions of 1.5 or 3.2 log CFU/cm^2, respectively, from PVC and 2.8 or 3.3 CFU/cm^2, respectively, from PS (**Fig. 5B**). Exposure to 460 nm caused the following reductions for the same two surface materials: 1.8–2.2 log CFU/cm^2 (PVC) and 2.7–2.9 CFU/cm^2 (PS) (**Fig. 5C**). It is worth noting here that 24 h exposures were not significantly different from 16 h exposures. To conclude, although some enhancement in viability reduction was observed after extending 405 nm exposures (Fig. 5A), this was not the case for other wavelengths (Fig. 5B-C), confirming that 16 h exposures, delivering different doses, attained consistent *Listeria* dried cells inactivation on all surfaces tested.

Objective 3

The specific aims were to 1) evaluate the application of high intensity lamps, and 2) assess the application on objects that simulate packing plant surfaces.

The treatments with the high intensity Panacol-USA lamp provided an opportunity to treat surfaces from a larger distance because of the higher irradiation doses. Because of the heating effect of high-power emissions, three different conditions were tested. At 405 nm, the treatment that achieved the greatest viability reduction, from 3 to 4.5 log CFU/cm^2 on SS and HDPE surfaces, respectively, was the 5 h treatment with 68 cycles of 40 seconds exposure between off-periods of cooling (**Table 1**). Surprisingly, reductions of 2.0 log CFU/cm^2 were even observed on the roller brushes. When greater than a meter of separation from the 405 nm lamp was used, reductions of more than 1.5 CFU/cm^2 were observed on metal and plastic surfaces.

Outcomes and Accomplishments

Results obtained from this initial study provide the first evidence that aBL can reduce the viability of *L. monocytogenes* on multiple surfaces and suggest that this can be a new tool for the improvement of microbial safety in packing and processing plants. The series of research activities conducted during the duration of this grant were very close to the planned work in the original proposal. There were very few changes to the experimental worked proposed. The main difference was the use of lower cost aBL lamps for Objectives 1 and 2.

The specific accomplishments were the following:

1. Completed the selection of *L. monocytogenes* strains for use in this project.
2. All experiments testing treatment of *L. monocytogenes* dried cells on stainless steel were completed, identifying optimal doses at three different wavelengths.
3. Planned evaluation of gallic acid as a photosensitizer was finished.
4. Treatment of biofilms on stainless steel at all three wavelengths was conducted.
5. Comparison of *L. monocytogenes* survival on different materials was carried out at all three aBL wavelengths.
6. Evaluation of high intensity aBL at 405 nm was completed on various surfaces that simulate those used at packing plants.

Summary of Findings and Recommendations

The following were the most important findings:

1. Consistent inactivation of *Listeria monocytogenes* on all types of surfaces at any wavelength is the main finding of this project.
2. The results indicated that the viability of dried cells and biofilms of *L. monocytogenes* was consistently reduced by exposure to all aBL wavelengths on any surface tested.
3. The viability reduction was dependent on the irradiation dose, but it appeared to reach a maximum at constant dose.
4. Exposure at 405 nm blue light was more effective than treatment with 420 and 460 nm aBL lamps to inactivate *Listeria* cells and biofilms.
5. Experiments with gallic acid as a photosensitizer suggested that its effect enhancing blue light inactivation was not consistent and, in many cases, not different from controls.
6. Using 405 nm aBL, the viable counts of both dried cells and biofilms were reduced by 3 to 4 log CFU/cm^2 on stainless steel surfaces after exposure to 2,672 J/cm^2, 16 h.
7. Biofilm micrographs displayed extensive shifts in biofilm structure from live to dead cells after aBL treatment.
8. The rate of viability reduction was dependent on the type of surface. On polystyrene, *Listeria* cells were killed faster than on any other surface.
9. Reductions of 2 to 3 log CFU/cm^2 were observed on the SS surface on pilot plant equipment, 4.5 log CFU/cm^2 on plastic surfaces, and 1.5 log CFU/cm^2 on brush bristles.
10. Significant reductions at more than a meter of distance were observed with high intensity aBL lamps.

Acknowledgments

Thanks to the support from CPS and the Texas Department of Agriculture Specialty Crop Block Grant Program, we were able to obtain a NIFA/USDA grant to continue evaluating aBL on *Listeria* and *Salmonella* biofilms as well as on viral pathogens. This project is scheduled to start in June 2022.

APPENDICES

Publications and Presentations

Two abstracts were submitted for poster presentation at the 2022 IAFP annual meeting (Pittsburgh, July 31-Aug 3):

1. Inactivation of *Listeria monocytogenes* biofilms on stainless steel surfaces using blue light – Magdalena Olszewska, Govindaraj Dev Kumar, and Francisco Diez-Gonzalez
2. Blue light efficacy against *Listeria monocytogenes* dried on inert surfaces – Magdalena Olszewska, Govindaraj Dev Kumar, and Francisco Diez-Gonzalez

Budget Summary

Total funds awarded for the research project were $139,645. Of the ~$98,000 in total expenditures to date, the majority of funds were spent on salary and fringe benefits for the post-doc research assistant and for supplies. The remaining balance (~$46,000) was the result of the lower cost of equipment used for Objectives 1 and 2, and less charges for salary and fringe caused by early departure of the post-doc and less involvement of undergraduate students.

Figures 1–5 and Table 1 (see below)

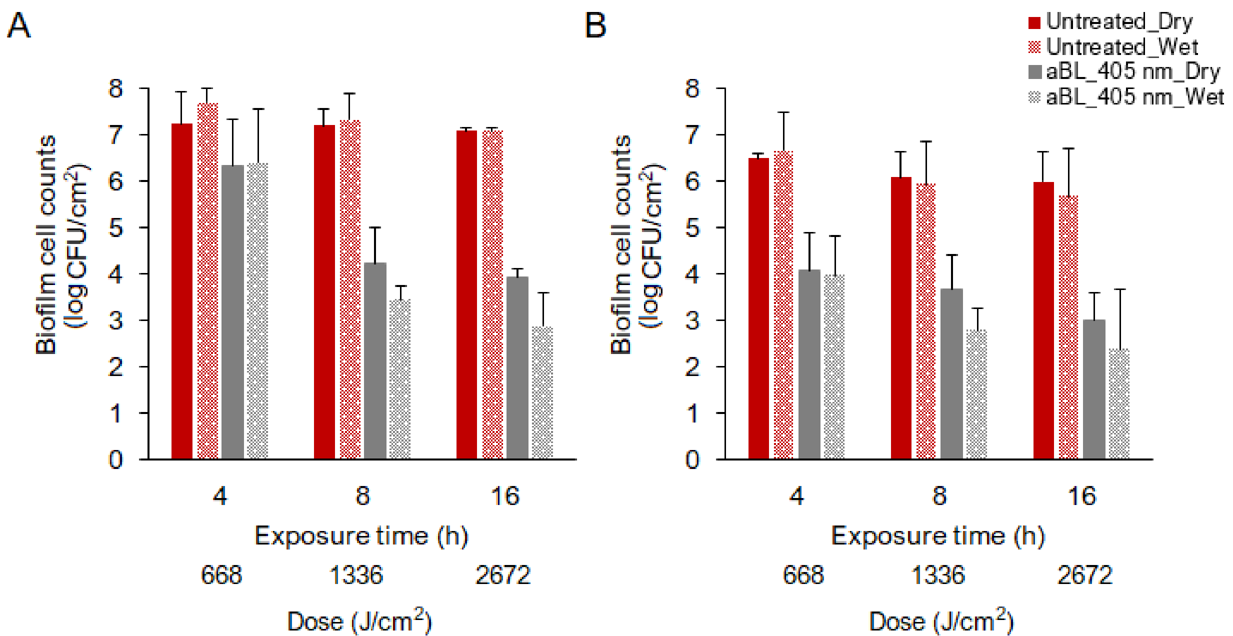

Figure 1. Effect of blue light at 405 nm on *Listeria monocytogenes* biofilms grown in full (A) and 1/10 (B) tryptic soy broth on stainless steel coupons. Biofilms were grown for 48 h. Coupons were prepared in duplicate where one series was dried before aBL exposure (dry biofilms) and the other not (wet biofilms). The bars represent the mean values ± standard deviations (n=9).

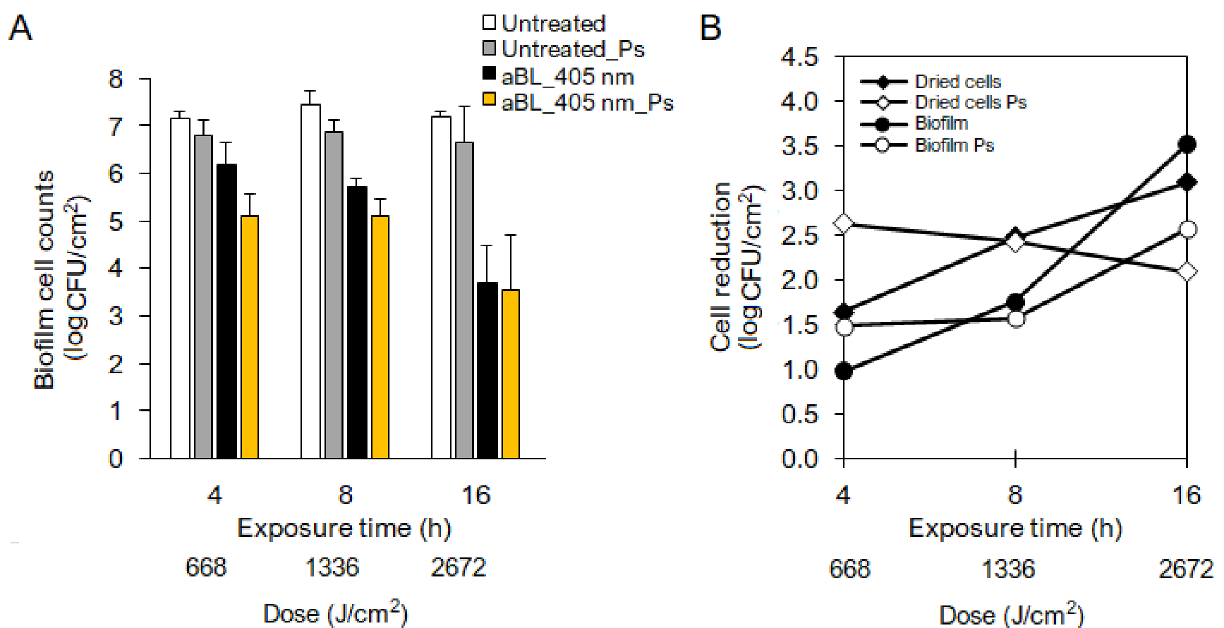

Figure 2. Effect of blue light at 405 nm on *Listeria monocytogenes* grown as biofilms on stainless steel coupons, alone and after the application of a photosensitizer (Ps), gallic acid (A). The bars represent the mean values ± standard deviations (n=9). Antimicrobial effectiveness of aBL and aBL with Ps for inactivation of *L. monocytogenes* dried cells and biofilms (B).

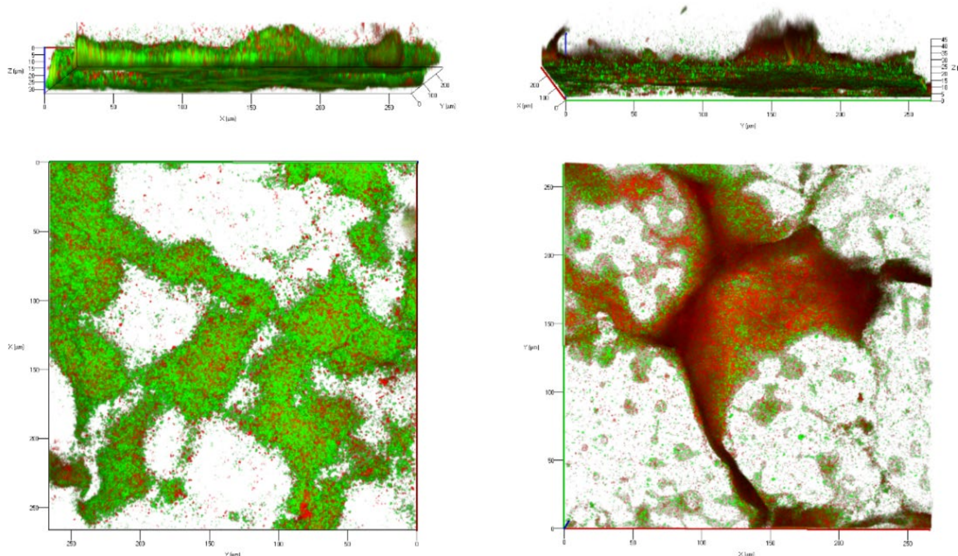

Figure 3. Three-dimensional images of control (left) and 405 nm (668 J/cm^2)-treated (right) *L. monocytogenes* biofilm. These images present the transparent and rotated projections obtained from confocal z-stacks using ZEN 2.3 software. The biofilm was grown in an eight-well chamber slide system in full tryptic soy broth for 48 h at 100 RPM and labelled with Syto® 9 and propidium iodide, wherein cells were visualized based on cell membrane integrity.

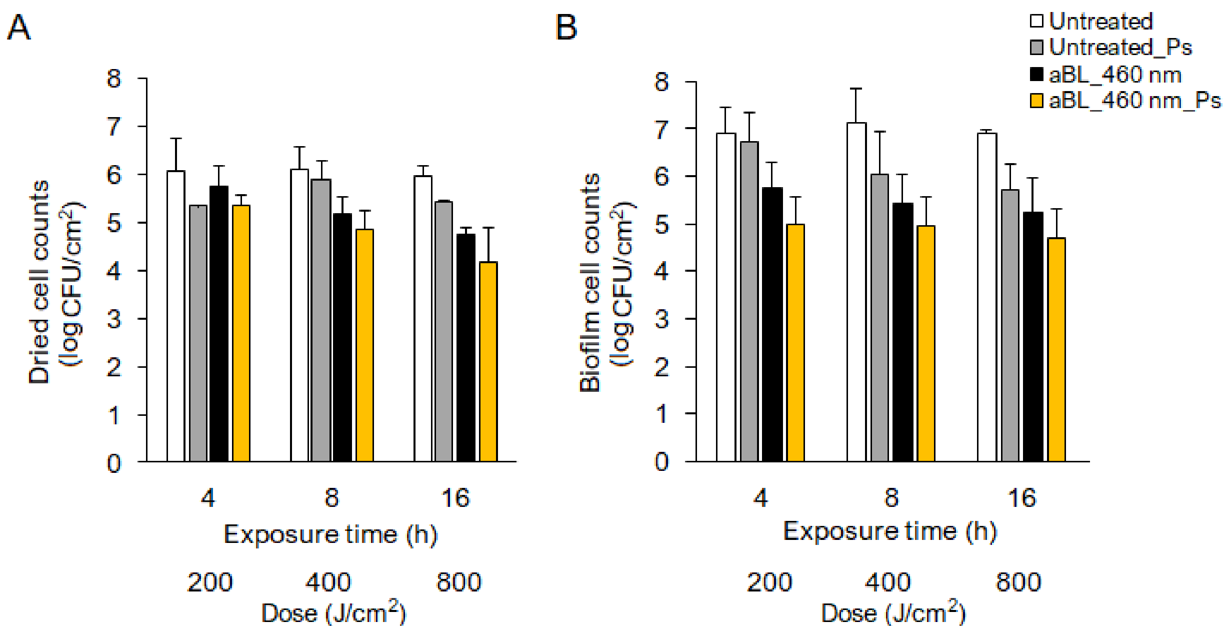

Figure 4. Effect of blue light at 460 nm on *Listeria monocytogenes* inoculated at high cell levels and dried (A) and grown as biofilms (B) on stainless steel coupons, alone and after the application of a photosensitizer (Ps), gallic acid. The bars represent the mean values ± standard deviations (n=9).

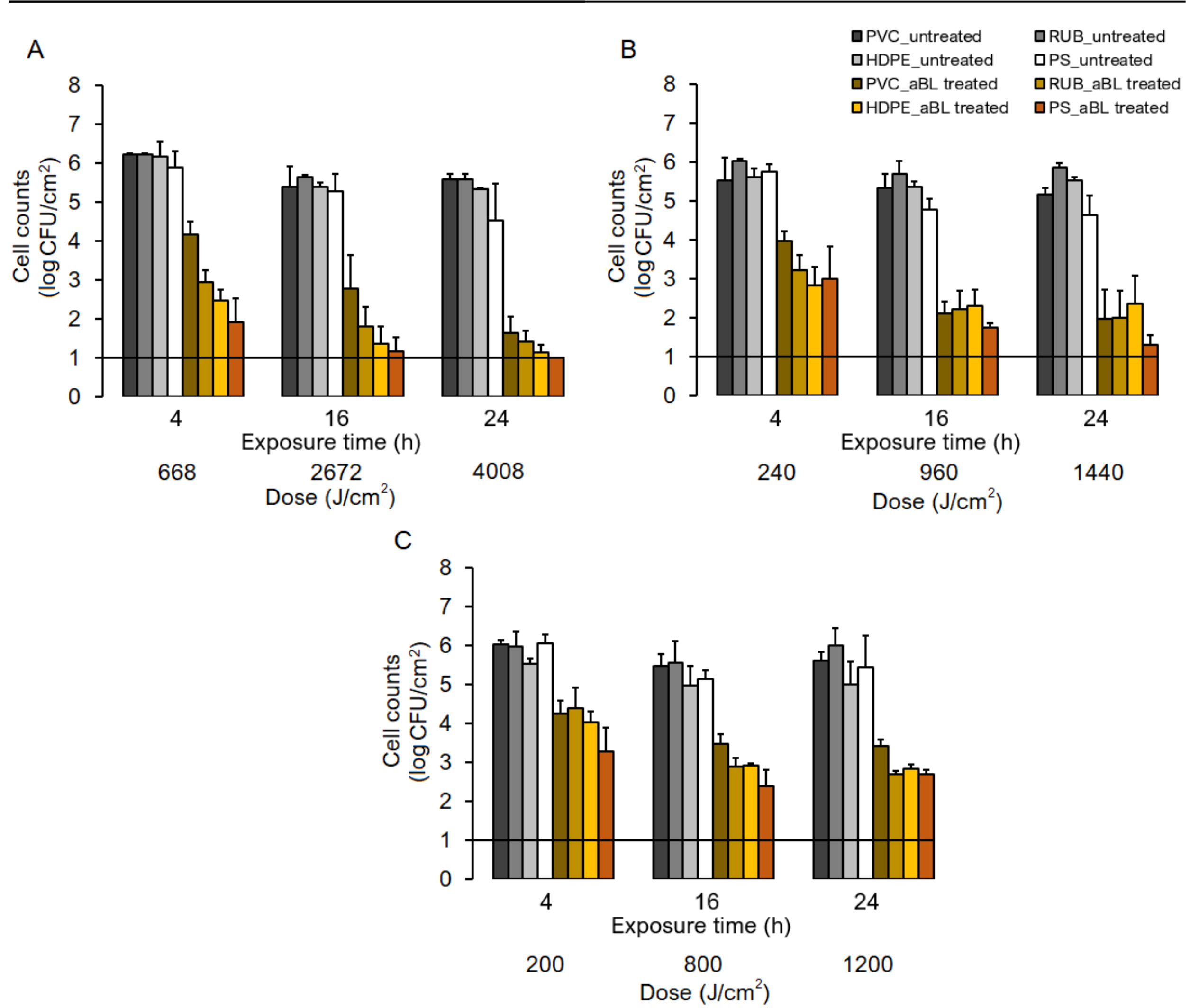

Figure 5. Effect of blue light at 405 nm (A), 420 nm (B), and 460 nm (C) on *Listeria monocytogenes* inoculated at high cell levels and dried on coupons made of different materials: polyvinyl chloride (PVC), silicone rubber (RUB), high-density polyethylene (HDPE), polystyrene (PS). The bars represent the mean values ± standard deviations (n=9).

Table 1. Reduction of viable cell count of *Listeria monocytogenes* inoculated onto four different surfaces resembling the packing plant environment: stainless steel (SS) trays, SS surface of pilot plant equipment, rigid HDPE plates, and black roller brush segments. Average ± standard deviation values of two independent experiments are shown.

Wave-length (nm)	Treatment	Dose (J/cm^2)	Viability reduction on each material (CFU/cm^2)			
			SS trays	SS equipment	HDPE plates	Roller brushes
405	25 cm / 2 h / temp control	101	1.81 ± 0.18	ND	ND	ND
	102 cm / 18 h	740	1.50 ± 0.25	ND	1.76 ± 0.24	0.47*
	9 cm / 68 cycles (40 s ON, 3 min OFF)	1660	3.07 ± 0.32	3.11 ± 0.04	4.50 ± 0.40	2.00 ± 0.18
460	102 cm / 18 h	415	1.32 ± 0.12	ND	1.15*	ND

ND, not determined.
*, only one experiment was performed.

References Cited

1. Zhu Q, Gooneratne R, Hussain M. 2017. *Listeria monocytogenes* in fresh produce: outbreaks, prevalence and contamination levels. Foods 6:21.
2. Angelo KM, Conrad AR, Saupe A, Dragoo H, West N, Sorenson A, Barnes A, Doyle M, Beal J, Jackson KA, Stroika S, Tarr C, Kucerova Z, Lance S, Gould LH, Wise M, Jackson BR. 2017. Multistate outbreak of *Listeria monocytogenes* infections linked to whole apples used in commercially produced, prepackaged caramel apples: United States, 2014-2015. Epidemiol Infect 145:848–856.
3. Chen Y, Burall LS, Luo Y, Timme R, Melka D, Muruvanda T, Payne J, Wang C, Kastanis G, Maounounen-Laasri A, De Jesus AJ, Curry PE, Stones R, K'Aluoch O, Liu E, Salter M, Hammack TS, Evans PS, Parish M, Allard MW, Datta A, Strain EA, Brown EW. 2016. *Listeria monocytogenes* in stone fruits linked to a multistate outbreak: Enumeration of cells and whole-genome sequencing. Appl Environ Microbiol 82:7030–7040.
4. CDC. 2021. Listeria Outbreak Linked to Queso Fresco Made by El Abuelito Cheese Inc. List Outbreaks.
5. Lin JT, Cheng DC, Chen KT, Liu HW. 2019. Dual-wavelength (UV and blue) controlled photopolymerization confinement for 3D-printing: Modeling and analysis of measurements. Polymers (Basel) 11:1–16.
6. Jung WS, Chung IM, Hwang MH, Kim SH, Yu CY, Ghimire BK. 2021. Application of light-emitting diodes for improving the nutritional quality and bioactive compound levels of some crops and medicinal plants. Molecules 26.
7. Wang YW, Ludwig HD, Scherm H, van Iersel MW, Nambeesan SU. 2020. Blue light does not affect fruit quality or disease development on ripe blueberry fruit during postharvest cold storage. Horticulturae 6:1–14.
8. Ploydaeng M, Rajatanavin N, Rattanakaemakorn P. 2021. UV-C light: A powerful technique for inactivating microorganisms and the related side effects to the skin. Photodermatol Photoimmunol Photomed 37:12–19.
9. Dai T, Gupta A, Murray CK, Vrahas MS, Tegos GP, Hamblin MR. 2012. Blue light for infectious diseases: *Propionibacterium acnes*, *Helicobacter pylori*, and beyond? Drug Resist Updat 15:233–236.
10. Hope CK, Strother M, Creber HK, Higham SM. 2016. Lethal photosensitisation of *Prevotellaceae* under anaerobic conditions by their endogenous porphyrins. Photodiagnosis Photodyn Ther 13:344–346.
11. Bumah V V., Masson-Meyers DS, Enwemeka CS. 2015. Blue 470 nm light suppresses the growth of *Salmonella enterica* and methicillin-resistant *Staphylococcus aureus* (MRSA) in vitro. Lasers Surg Med 47:595–601.
12. Abana CM, Brannon JR, Ebbott RA, Dunigan TL, Guckes KR, Fuseini H, Powers J, Rogers BR, Hadjifrangiskou M. 2017. Characterization of blue light irradiation effects on pathogenic and nonpathogenic *Escherichia coli*. Microbiol. 6:1–10.
13. Roh HJ, Kim A, Kang GS, Kim DH. 2016. Photoinactivation of major bacterial pathogens in aquaculture. Fish Aquat Sci 19:1–7.
14. Halstead FD, Thwaite JE, Burt R, Laws TR, Raguse M, Moeller R, Webber MA, Oppenheim BA. 2016. Antibacterial activity of blue light against nosocomial wound pathogens growing planktonically and as mature biofilms. Appl Environ Microbiol 82:4006–4016.
15. Maclean M, MacGregor SJ, Anderson JG, Woolsey GA, Coia JE, Hamilton K, Taggart I, Watson SB, Thakker B, Gettinby G. 2010. Environmental decontamination of a hospital isolation room using high-intensity narrow-spectrum light. J Hosp Infect 76:247–251.
16. Bache SE, MacLean M, MacGregor SJ, Anderson JG, Gettinby G, Coia JE, Taggart I. 2012. Clinical studies of the high-intensity narrow-spectrum light environmental

decontamination system (HINS-light EDS), for continuous disinfection in the burn unit inpatient and outpatient settings. Burns 38:69–76.

17. Tomb RM, Maclean M, Coia JE, MacGregor SJ, Anderson JG. 2017. Assessment of the potential for resistance to antimicrobial violet-blue light in *Staphylococcus aureus*. Antimicrob Resist Infect Control 6:1–13.
18. Endarko E, Maclean M, Timoshkin I V., MacGregor SJ, Anderson JG. 2012. High-intensity 405 nm light inactivation of *Listeria monocytogenes*. Photochem Photobiol 88:1280–1286.
19. Murdoch LE, MacLean M, Endarko E, MacGregor SJ, Anderson JG. 2012. Bactericidal effects of 405nm light exposure demonstrated by inactivation of *Escherichia*, *Salmonella*, *Shigella*, *Listeria*, and *Mycobacterium* species in liquid suspensions and on exposed surfaces. Sci World J.
20. Kim MJ, Mikš-Krajnik M, Kumar A, Ghate V, Yuk HG. 2015. Antibacterial effect and mechanism of high-intensity 405 ± 5 nm light emitting diode on *Bacillus cereus*, *Listeria monocytogenes*, and *Staphylococcus aureus* under refrigerated condition. J Photochem Photobiol B Biol 153:33–39.
21. O'Donoghue B, NicAogáin 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 σ band the blue-light sensor Lmo0799. Appl Environ Microbiol 82:4017–4027.
22. Josewin SW, Kim MJ, Yuk HG. 2018. Inactivation of *Listeria monocytogenes* and *Salmonella* spp. on cantaloupe rinds by blue light emitting diodes (LEDs). Food Microbiol 76:219–225.