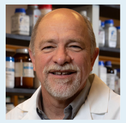
Control of *Listeria monocytogenes* in processing/packing plants using antimicrobial blue light (aBL)



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Summary

Antimicrobial blue light (aBL) in the 400–470 nm range has been reported to kill a wide range of microbes. This project evaluated the efficacy of antimicrobial blue light (aBL) to reduce the viability of *Listeria monocytogenes* on stainless steel (SS) coupons. Dried cells and biofilms of five-strain mixtures were treated at 405, 420 and 460 nm. Different radiation doses, inoculation levels and the use of gallic acid as a photosensitizer were applied and the viable counts were determined. Biofilms were observed with fluorescence microscopy. aBL exposure at 405, 420 and 460 nm caused reductions of approximately 3.0, 1.0 and 1.2 log CFU/cm^2, respectively, using 7488, 1382, and 749 Joules/cm^2. Gallic acid enhanced inactivation. Micrographs showed extensive bacterial cell damage in biofilms.

Methods

Several emission doses from three aBL lamps (405, 420 and 460 nm) were tested at low and high inoculums (3 and 6 log CFU/cm², respectively) of 5-strain cocktails inoculated and dried on SS coupons, alone and after the application of a photosensitizer (gallic acid; 10 mM). Cells were removed by sonication for a duration of 1 min, and counts were measured after standard dilution and plating on tryptic soy agar (TSA), followed by incubation at 37°C for 48 h. Biofilm response to aBL was investigated on SS coupons after cultivating biofilms for 48 h in TSA by standard microbiological methods and confocal laser scanning microscopy (CLSM) with Syto® 9 and propidium iodide staining. Two independent experiments were conducted in triplicate and subjected to statistical analysis.

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.
   - The specific aims of Objective 1 were to i) assess the aBL antimicrobial effect at three wavelengths at several doses, ii) determine the influence of inoculation level and application of a photosensitizer, iii) compare the effect on dried cells and biofilms, and iv) characterize the aBL effect on biofilm viability and structure.
2. Evaluate antimicrobial blue light (aBL) efficacy for *Listeria* reduction on processing-related surfaces.
3. Assess aBL decontamination effectiveness at a pilot-plant scale.

Results to Date

At 405 nm, the counts of dried cells inoculated onto SS coupons (2672 J/cm²,16 h) were reduced by 3.1 log CFU/cm² ($P < 0.05$; **Figure 1**). Gallic acid application resulted in an additional 1 log CFU/cm² at 668 J/cm². Similar effects were observed with the low inoculum.

At 420 nm, a maximum non-significant reduction of 1.0 log was observed at 473 J/cm² (data not shown). At 460 nm, viable counts were reduced from 6 to 4.8 log CFU/cm² after exposure to 272 J/cm² (**Figure 2**). Wet and dry biofilms treated at 405 nm had from 3 to 4.1 log CFU/cm² less viable cells at 1336 and 2672 J/cm² (**Figure 3**). Biofilm micrographs displayed extensive shifts in biofilm structure from live to dead cells after aBL treatment (**Figure 4**).

Benefits to the Industry

Results obtained from this initial study provide the first evidence that aBL is capable of reducing the viability of *L. monocytogenes* on SS surfaces, and suggest that this can be a new tool for the improvement of microbial safety in packing and processing plants. The aBL technology could be environmentally friendly, safe, and adaptable to different production systems. The use of aBL for mitigation of *L. monocytogenes* will 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.

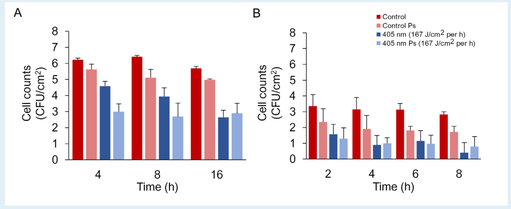

Figure 1. Effect of blue light at 405 nm on *Listeria monocytogenes* inoculated at high (A) and low (B) cell levels and dried on stainless steel coupons, alone and after the application of gallic acid (10 mM), a photosensitizer (Ps). The bars represent the mean values ± standard deviations (n=9).

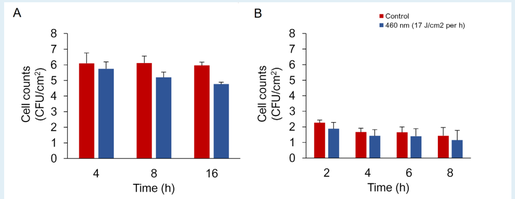

Figure 2. Effect of blue light at 460 nm on *Listeria monocytogenes* inoculated at high (A) and low (B) cell levels and dried on stainless steel coupons, alone. The bars represent the mean values ± standard deviations (n=9).

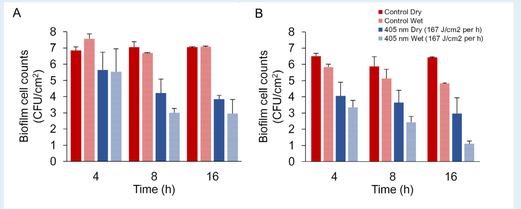

Figure 3. Effect of blue light at 405 nm on *Listeria monocytogenes* biofilms grown in full (A) and 1/10 (B) tryptic soy broth on stainless steel coupons. Biofilms were grown for 48 h. Coupons were prepared in duplicate, where one series was dried before aBL exposure and another was exposed to aBL immediately after growth and washing. The bars represent the mean values ± standard deviations (n=9).

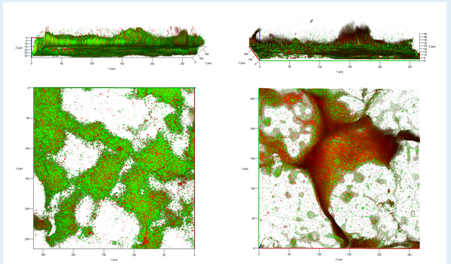

Figure 4. Three-dimensional images of control (left) and 405 nm (668 J/cm2)-treated (right) *L. monocytogenes* biofilm. These images present the transparent and rotated projections obtained from confocal z-stacks using ZEN 2.3 software. The biofilm was grown in an 8-well chamber slide system in full 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.