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Efficacy of Ultraviolet Light Exposure Against Survival of *Listeria monocytogenes* on Conveyor Belts

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Abstract

*Listeria monocytogenes* has been repeatedly isolated from foods and food-processing facilities including food contact surfaces such as conveyor belts (CB). CBs are often difficult to clean and require rigorous sanitation programs for decontamination. Ultraviolet (UV) light has exhibited microbicidal properties on food contact surfaces and this study was conducted to determine the efficacy of UV against *L. monocytogenes* on CB made of different materials. A four-strain cocktail of *L. monocytogenes* (serotypes 3A, 4A, 4B, and 4C) was made to give a suspension of approximately $10^7$ CFU/mL. CBs made from four different types of materials, (1) Ropanyl DM 8/2 A2+04 (belt 1), (2) Volta FRMW-3.0 (belt 2), (3) Volta FRMB-3.0 (belt 3), and (4) Ropanyl DM (belt 4), were inoculated with 1 mL of the four-strain cocktail ($\sim 10^7$ CFU/mL) of the bacterial suspension. CBs were treated with UV light (254 nm) for 1 and 3 sec at 5.53 and 5.95 mW/cm². Three replications of the experiments were conducted. Two-way analysis of variance of survival populations of *L. monocytogenes* showed that bacterial counts were significantly reduced ($p < 0.05$) on all belt types irrespective of UV light intensities and times of exposure. *L. monocytogenes* populations were reduced ($p < 0.05$) to below detection limits on belts 1, 2, and 3 after exposure to 5.95 mW/cm² UV light intensity for 3 sec. *L. monocytogenes*–inoculated CBs that were exposed to 5.53 mW/cm² showed higher ($p < 0.05$) survival populations of *L. monocytogenes* compared with 5.95 mW/cm² on all the four CBs. Belt 4 showed survival populations of *L. monocytogenes* ranging from 1.42 to 1.73 log10 CFU/cm² after UV light treatment for 1 and 3 sec. UV light can be effectively used to reduce *L. monocytogenes* contamination on CBs.

Introduction

*Listeria monocytogenes* is a major foodborne pathogen associated with ready-to-eat foods. According to the Centers for Disease Control and Prevention (CDC, 2005) *L. monocytogenes* causes nausea, vomiting, fever, meningencephalitis, stillbirths, and abortions, and can be fatal to pregnant women, immunocompromised individuals, elderly, and infants. Hence, the U.S. Department of Agriculture has a “zero tolerance” policy toward *L. monocytogenes* in ready-to-eat foods. *Listeria* enters food-processing environments through raw material and soil. Although the optimal growth temperatures are between 30°C and 37°C, *Listeria* are capable of growth over a temperature range of −0.4°C to 50°C, making the organism a potential food safety concern in refrigerated/cold environments (Johnson et al., 1990). The food hazards posed by *L. monocytogenes* are especially due to its ability to grow over a broad temperature range, a characteristic enabling the pathogen to modify its membrane composition to maintain membrane fluidity (Lado and Yousef, 2007). It survives and grows on food contact surfaces like conveyor belts (CBs) and can secrete extracellular polysaccharides to adhere and form biofilms. These biofilms are difficult to clean especially from surfaces like CBs due to their intricate design and are subsequently disseminated onto foods during processing. Removal of bacterial cells and their biofilms is a difficult and demanding task, and current sanitation programs are usually not sufficient. Thus, the food industry has been looking for efficient cleaning and sanitation alternatives to prevent attachment of pathogenic bacteria to food contact surfaces and potentially even biofilms. These new strategies usually include physical and chemical methods that interfere with bacterial attachment, colonization, and potential biofilm development. Most of the preceding research on the attachment of *Listeria* to inert surfaces has been done on a few classic materials such as stainless steel (Beresford et al., 2001).

Bactericidal effects of ultraviolet (UV) light have long been utilized in the medical and some food industry areas but only recently developed for use on foods such as surfaces of meat. As a result of this, UV can be used as an alternative to remove...
biofilms and bacteria from CBs. UV generally ranges from 100 to 380 nm, but the germicidal activity is seen from 200 to 280 nm. UV forms pyrimidine dimers in the genomic DNA that affects cell functions like protein synthesis, and once these pyrimidine dimers reach a certain level, cell death occurs (Eischied and Linden, 2007). The use of UV light is well documented for water treatment, air disinfection, and decontamination of smooth surfaces such as stainless steel in bakeries, cheese, and dairy plants (Koutchma, 2008). Although UV light can be used in food processing as it is a nonthermal, environmentally friendly microbicidal agent that does not leave any residue in foods (Guerrero-Beltran and Barbosa-Canovas, 2004), it can potentially be harmful for workers in the processing plant if adequate safety measures are not taken. It has been used to disinfect chicken skin (Sumner et al., 1996), skinless chicken breast meat (Lyon et al., 2007), eggs (Coufal et al., 2003), and packaging material (Silva et al., 2003). Despite the efficacy of UV light to disinfect smooth surfaces, interactions between the microorganisms and surface materials, and structure and topography of surface materials may impact the efficacy of UV light (Koutchma, 2008). There is limited literature on the use of UV to disinfect CBs. Hence, the objective of this study was to determine the efficacy of UV light on the survival of L. monocytogenes on different types of CBs.

Materials and Methods

Bacterial culture

L. monocytogenes serotypes 3A (culture # 2298, KC # 1708 obtained from NADC, Ames, IA), 4A (culture #2164 obtained from NADC), 4B (culture # 2045 obtained from NADC), and 4C (ATCC #19116 obtained from NADC) were grown for 12 and 24 h to determine efficacy of UV light on cultures at different phases of growth. All cultures were grown in brain heart infusion (Difco, Detroit, MI) broth incubated at 37°C for either 12 or 24 h before exposure to UV light. As no significant differences (p > 0.05) were observed between the 12 and 24 h cultures (data not shown), all strains were grown for 24 h in brain heart infusion and incubated at 37°C for further experiments. A four-strain cocktail was made to obtain a final concentration of 10^7 CFU/mL before inoculating the CBs.

Conveyor belts

Belts (Ammeraal Beltech, Heerhugowaard, The Netherlands) made from four different materials were used in this study: Ropanyll DM 8/2 A2+04 Light Blue thermoplastic polyurethane (belt 1); Volta FRMW-3.0 Beige CEB thermoplastic elastomer beige (belt 2); Volta FRMB-3.0 Blue CEB thermoplastic elastomer blue (belt 3); and Ropanyll DM thermoplastic polyurethane 04+04 White food-grade Amerol (belt 4).

Inoculation of CBs

CBs were cut into 7×2 cm pieces and then sterilized using 90% ethanol. Sterilized pieces of the CBs were then inoculated with a 1 mL (~7 log_{10} CFU/mL) cocktail of L. monocytogenes that was spread using a sterile cotton swab. These inoculated CB pieces were then dried under a biological safety hood (Labgard 437; NuAire Inc., Plymouth, MN) for 30 min to allow bacterial attachment. The CB pieces were randomly assigned to UV treatment and the nontreated (control) pieces were swabbed to determine levels of L. monocytogenes.

UV exposure

UV light source consisted of a panel of 10 UV bulbs (254 nm). The intensity of UV light was measured by the UVX intensity meter (Ultraviolet Products, Upland, CA), and the inoculated dry pieces of the four different CBs were exposed to UV light intensities of 5.53 and 5.95 mW/cm² for 1 and 3 sec. Similarly, 12- and 24-h-old cultures inoculated onto the CBs were exposed to UV light intensities of 5.53 and 5.95 mW/cm² for 1 and 3 sec.

Bacterial enumeration

Nontreated CB pieces were swabbed and placed in 9 mL of 0.1% peptone water (Difco, Sparks, MD), serially diluted and plated onto modified Oxford agar (MOX; Oxoid Ltd., Basingstoke, Hampshire, United Kingdom), and incubated at 37°C for 24 h, and populations of L. monocytogenes were enumerated to represent the controls. Survival populations of L. monocytogenes were determined by swabbing the entire surface of the CBs. The swabs were placed in 9 mL of 0.1% peptone water and serially diluted, and 0.1 mL was spread onto MOX. The MOX plates were then incubated at 37°C for 36 h, and survival populations of L. monocytogenes were reported as log_{10} CFU/cm².

Statistics

All experiments were performed in triplicates on separate days and considered as independent replications. Data obtained were analyzed using General Linear Models procedures of SAS with two doses (UV intensities)×two exposure times (1 and 3 sec)×four CB materials. Two-way analysis of variance was conducted and the least square means of survival populations of L. monocytogenes were differentiated at α = 0.05. All counts below the detection limit were recorded as 0.5 log_{10} CFU/cm² (equivalent to 3.2 CFU/cm²) for statistical analysis.

Results and Discussion

Analysis of variance of survival populations of L. monocytogenes (log_{10} CFU/cm²) indicated that there was no significant (p > 0.05) differences between the 12- and 24-h-old cultures after exposure to UV light at 5.53 and 5.95 mW/cm² for either 1 or 3 sec (data not shown), suggesting that age of the culture did not affect sensitivity to UV exposure. These results are in agreement with those reported by Yousef and Marth (1988), who reported no differences in UV sensitivity of 24- and 48-h-old Listeria cells. As no significant differences were observed on the efficacy of UV light against 12- and 24-h-old cultures of L. monocytogenes, further experiments were conducted using 24-h-old cultures for inoculation of the CBs.

Exposure of belt 1 to 5.53 and 5.95 mW/cm² showed a significant (p < 0.05) reduction in the bacterial population (Table 1). L. monocytogenes populations were reduced to below the detection limit of 3.2 CFU/cm² after 1 and 3 sec of exposure at 5.95 mW/cm², whereas at 5.53 mW/cm² the survival populations were 1.31 and 0.74 log_{10} CFU/cm² after 1 and 3 sec of exposure on belt 1, respectively. Similar significant (p < 0.05)
reductions were observed on belts 2 and 3 with survival populations of 2.38 and 1.31 log_{10} CFU/cm^2 at 5.53 mW/cm^2 of exposure for 1 and 3 sec, respectively, on belt 2, and 1.33 log_{10} CFU/cm^2 after 1 sec of exposure at 5.95 mW/cm^2 and no detectable survival populations after 3 sec of exposure at 5.95 mW/cm^2 on belt 2 (Table 1). On belt 3, UV light intensity of 5.53 mW/cm^2 resulted in 2.04 log_{10} CFU/cm^2 survival populations of *L. monocytogenes* after 1 sec, whereas no survival populations were detected after 3 sec of exposure (Table 1). Exposing belt 3 to 5.95 mw/cm^2 resulted in 1.63 log_{10} CFU/cm^2 survival populations of *L. monocytogenes* after 1 sec of exposure, whereas no survival was detected after 3 sec of exposure. On belt 4, there was a significant difference in the survival populations of *L. monocytogenes* (Table 1) compared with other belts after 3 sec of exposure with a 1.73 and 1.42 log_{10} CFU/cm^2 survival at 5.53 and 5.95 mW/cm^2 exposure levels, respectively. This increased survival on belt 4 can be attributed to the rough surfaces that could potentially help in shielding the bacteria from UV light as compared to belts 1, 2, and 3, which had visually smooth surfaces that would offer less protection from UV light.

Since UV lacks penetration capability, the cracks and protuberances on rough surface can protect *L. monocytogenes* against UV (Silva et al., 2003), and similar observations regarding sample surface topography were made in a study by Kim et al. (2002) in which they inoculated stainless steel chips and chicken meat with or without skin with *L. monocytogenes*, *Salmonella Typhimurium*, and *Escherichia coli*. In their study Kim et al. (2002) reported 2.43 and 2.91 log_{10} CFU/cm^2 reduction of *L. monocytogenes* population after 1 min exposure of inoculated stainless steel coupons to 250 and 500 mW/cm^2, respectively. Although, the researchers found a significant reduction in *L. monocytogenes* levels on smooth stainless steel surface, UV exposure of chicken meat with and without skin resulted in only a 0.25 and 0.13 log_{10} CFU/cm^2 reduction on *L. monocytogenes* at 500 mW/cm^2 for 1 min. In addition, Silva et al. (2003) also reported a reduced efficacy of UV light in reducing populations of *Staphylococcus aureus* and *E. coli* due to the cracks and crevices found on low-density polyethylene film. Results from our study have shown that exposure times as short as 1 and 3 sec are effective in reducing populations of *L. monocytogenes*, suggesting a potential application for sanitation of moving CBs in a processing plant. Thus, flash methods such as pulsed UV light can be used for the treatment of surfaces to eliminate/reduce bacterial populations, and the surface topography is an important factor that can effect the efficiency of UV light to reduce populations of *L. monocytogenes*.

### Conclusions

Results from this study showed that UV light can reduce *L. monocytogenes* on CBs but the degree of reduction is dependent on the type of belt material. Although UV light can reduce surface contamination, difference in bactericidal effects of UV due to materials and topographical variations of CBs should be taken into account to develop an effective in-process CB UV sanitation system.

### Disclosure Statement

No competing financial interests exist.

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