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# Antimicrobial coatings for controlling *Listeria monocytogenes* based on polylactide modified with titanium dioxide and illuminated with UV-A

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ABSTRACT

The anti-listerial properties of biodegradable polylactide coatings modified with titanium dioxide have been studied. Free standing films were prepared by casting solutions prepared from titanium dioxide and previously extruded polylactide. It was demonstrated that polylactide alone could support 2.84 ± 0.10 log CFU reduction of *Listeria monocytogenes* when incubated at 23 °C for 2 h. However, the log reduction for *Listeria* could be increased to >4 log CFU with titanium dioxide:polylactide composites illuminated with UV-A. The inactivation kinetics of *L. monocytogenes* followed a diphasic die-off with an initial 30 min lag period then a progressive decline in bacterial levels over a further 90 min period. The anti-listeria effect of polylactide:titanium dioxide films was dependent on illumination with UV-A but independent on the concentration of $TiO_2$ incorporated in the film within the range of 1–5% w/w. The mode of *L. monocytogenes* inactivation was via direct contact of the pathogen with the polylactide, in addition to the generation of oxygen radicals produced by excitation of the titanium dioxide. The composite film illuminated with UV-A was equally effective against *Salmonella* Typhimurium and Shiga toxin producing *Escherichia coli*. The coating was stable to 5 repeated sanitation cycles consisting of detergent and sodium hypochlorite rinses. The polylactide-titanium dioxide coating shows potential as an antimicrobial coating although further work is required to assess if the protective film can function under commercial conditions.



## 1. Introduction

*Listeria monocytogenes* is a highly virulent pathogen that can have a mortality rate of up to 30% amongst high risk groups such as the young, old, pregnant and immune-compromised (Warriner & Namvar, 2009). Previously, *L. monocytogenes* was primarily associated with deli meats and soft cheese but in recent times a greater diversity of food vehicles have been implicated in recalls and outbreaks (Burall, Grim, Mammel, & Datta, 2016; Salazar et al., 2016). For example, significant recalls have been issued for leafy greens, frozen vegetables and sesame seeds. Although *L. monocytogenes* is widely distributed in the natural environment the most significant source originates from endemic populations within the processing plant (Keskinen, Todd, & Ryser, 2008b; Thomas et al., 2015). For example, a listeriosis outbreak of 2008 linked to deli meats was traced to a contaminated slicing machine that had not been effectively sanitized over a number of years (Thomas et al., 2015). In the case of the listeriosis outbreak of 2011 associated with cantaloupes, there was a high prevalence of *L. monocytogenes* isolated in the processing environment (Martinez, Osborne, Jayeola, Katic, & Kathariou, 2016). Indeed, in many outbreaks it has been demonstrated that *L. monocytogenes* can become established in processing environments thereby representing a continuous source of contamination (Magalhaes et al., 2016).

Although having an effective sanitation plan can reduce the prevalence of *L. monocytogenes* the prevention of biofilms remains a significant challenge (de Candia, Morea, & Baruzzi, 2015; Keskinen, Todd, & Ryser, 2008a). It is for this reason there has

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been interest in developing temporary or permanent antimicrobial coatings for food contact and non-contract surfaces to prevent colonization of pathogens such as *L. monocytogenes* (Bastarrachea, Denis-Rohr, & Goddard, 2015). There has been sustained interest in developing antimicrobial coatings, with those based on titanium dioxide attracting most attention (Kugel, Stafslien, & Chisholm, 2011; Salwiczek et al., 2014). Anatase titanium dioxide is widely used as, for example, a photocatalyst for self-cleaning and antimicrobial surfaces (Dural-Erem, Erem, Ozcan, & Skrifvars, 2015; Han, Lalley, Namboodiri, Cromer, & Nadagouda, 2016; Hashimoto, Irie, & Fujishima, 2005). The underlying mechanism relies on formation of radicals from the breakdown of water and oxygen following illumination with UV light in the range of 254–395 nm. UV-A is commonly used in the excitation of titanium dioxide due to the working environment hazards associated with UV-C.

Titanium dioxide has been introduced within a range of polymer bases that retain the antimicrobial agent and also provides mechanical robustness to the coating. In a previous example, polyurethane modified with titanium dioxide was assessed for inactivating surface inoculated with *L. monocytogenes*, *Salmonella*, *Pseudomonas aeruginosa* and *Escherichia coli* (Weng, van Niekerk, Neethirajan, & Warriner, 2016). Although the surface had good durability, the log reductions of the aforementioned bacteria were limited to 0.5–1.0 log CFU despite illumination of the modified film with UV-A.

In the following, polylactide:titanium dioxide films were fabricated and evaluated for anti-listerial activity. Polylactide has received significant attention as a biodegradable linear aliphatic thermoplastic polyester with low toxicity and good processability (Fonseca et al., 2015). The mechanical properties of polylactide are compatible with surface coatings with respect to high tensile strength and Young's modulus giving rise to strong adhesion properties (Fonseca et al., 2015). A further advantage of polylactide films is that the polymer does not support strong bacterial attachment by virtue of the weak negative charge thereby minimizing biofilm formation (Wojciechowski & Klodzinska, 2015).

Polylactide modified with titanium dioxide has been described although limited to reporting the strong mechanical properties and processability of the polymer (Luo, Li, Wang, Xu, & Wang, 2009; Mhlanga & Ray, 2014; Zhang et al., 2015). The antimicrobial properties of polylactide:titanium dioxide composites have been less well studied. It has been reported that polylactide modified with titanium dioxide can inhibit the growth of *Klebsiella pneumoniae* and *Staphylococcus aureus* using an agar plate assay (Dural-Erem et al., 2015). Here, polylactide-$TiO_2$ films were overlaid onto agar plates inoculated with the test bacteria then incubated at 37 °C for 18 h to assess growth inhibiting effects. Although the agar plate assay is a useful preliminary screen, the technique does not provide insight into the microbial inactivation kinetics. Furthermore, there has been no studies to determine the antimicrobial properties of polylactide:titanium dioxide against pathogens of direct relevance to food safety, namely *L. monocytogenes*, *Salmonella* and *E. coli* O157:H7. In a further report, Fonseca et al., (2015) fabricated polylactide modified with titanium dioxide nanoparticles then assessed antimicrobial activity by submerging coupons in brain heart infusion broth inoculated with *E. coli* then illuminated with UV-A. The authors reported a 94% reduction on *E. coli* numbers compared to control cultures although it was unclear if this could be attributed to bacteriostatic or bacteriocidal activity (Fonseca et al., 2015). Consequently, the actual antimicrobial activity of polylactide:titanium dioxide films remains uncertain and requires to be investigated further. The following reports on the antimicrobial activity of polylactide films modified with titanium dioxide with respect to inactivation of *L. monocytogenes* and stability to sanitation cycles commonly used in food processing.

## 2. Materials and methods

### 2.1. Bacteria and cultivation conditions

*Listeria monocytogenes* 7163 Serotype 1/2a, *Salmonella enterica* subsp *enterica* Typhimurium DT104 and *Escherichia coli* O157:H7 ph 1 were individually cultivated in 30 mL tryptic soy broth at 37 °C for 24 h. The cells were harvested by centrifugation (10 min, 5000 rpm, 22 °C) and resuspended in saline to a final optical density at 600 nm of 2 (8–9 log CFU $mL^{-1}$). The suspensions were further diluted to 7 log CFU $mL^{-1}$ and used to inoculate the test films.

### 2.2. Fabrication of titanium dioxide modified polylactide films

Polylactide (PLA) films were prepared using 4032D poly(lactic acid) resin (Nature Works, Minnetoka, MN, USA) and a lab-scale extruder (Microtruder RCP-0625, Randcastle, NJ, USA). Film pieces (10 g) were cut and dissolved in 10 mL DMSO (Sigma-Aldrich, Oakville, Canada) along with 0.1% w/w titanium dioxide (20:80% rutile:anatase P25 grade <100 nm diameter; Degussa Corporation, NJ, USA). The sample was held within a sonication bath for 5 min to ensure homogenous distribution of the titanium dioxide before pouring the solution into a 90 mm glass petri dish. The solvent was evaporated overnight in a fume-hood and the formed film cut into 2 $cm^2$ sections of 30 ± 2 μm thickness.

The test bacteria were inoculated (0.1 mL of a 7 log CFU $mL^{-1}$ suspension) onto one side of the polylactide-titanium dioxide film and allowed to attach for 20 min. One set of films were placed under a UV-A lamp (Cole-Parmer, Montreal, Canada; 366 nm, 15 W placed at a 10 cm distance from the film surface) with samples ($n = 3$) being withdrawn periodically to determined number of survivors. Here, film sections were placed in 10 mL of sterile saline containing 1% Tween then vortexed for 30 s to release bacteria. A dilution series was prepared in saline then plated onto the appropriate selective media depending on the bacterial type. For *L. monocytogenes*, Modified Oxford Agar (Oxoid, Basingstoke, UK) was used and incubated at 30 °C for 48 h. *Salmonella* Typhimurium was enumerated on XLD agar (Oxoid) incubated at 37 °C for 24 h. *E. coli* O157:H7 was enumerated on CT-SMac (Oxoid) incubated at 37 °C for 24 h.

Polyurethane modified with 5% w/w titanium dioxide was supplied by Sun-Wash Technologies Inc (London, Ontario, Canada) (Weng et al., 2016).

### 2.3. Effect of sanitation cycles on anti-listerial activity

Polylactide:titanium dioxide film sections were subjected to sanitation cycles that consisted of dipping a set of three coupons in 10 mL sterile water for 30 s followed by a dip in 10 mL 0.1% non-ionic detergent (Thermo-Fisher, Whitby, Canada) for the same duration. The film sections were dipped for 30 s in 10 mL water then into the same volume of sodium hypochlorite solution (100 ppm free chlorine, pH 6.5) before a final water rinse (10 mL for 30 s). The films were then dried for 1 h and the process repeated for the designated number of cycles (up to 5). The antimicrobial activity of film sections were assessed by inoculating with *Listeria* and placing under UV-A light for 60 min before recovering survivors.

### 2.4. Experimental plan and statistics

Each experiment was repeated at least twice with three replicates per treatment being applied. The counts were converted to log values and compared using ANOVA and Tukey test.

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