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Inactivation of avirulent *Yersinia pestis* on food and food contact surfaces by ultraviolet light and freezing☆

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ABSTRACT

Yersinia pestis, the causative agent of plague, can occasionally be contracted as a naso-pharyngeal or gastrointestinal illness through consumption of contaminated meat. In this study, the use of 254 nm ultraviolet light (UV-C) to inactivate a multi-isolate cocktail of avirulent *Y. pestis* on food and food contact surfaces was investigated. When a commercial UV-C conveyor was used (5 mW/cm^2/s) 0.5 J/cm^2 inactivated >7 log of the *Y. pestis* cocktail on agar plates. At 0.5 J/cm^2, UV-C inactivated ca. 4 log of *Y. pestis* in beef, chicken, and catfish, exudates inoculated onto high density polypropylene or polyethylene, and stainless steel coupons, and >6 log was eliminated at 1 J/cm^2. Approximately 1 log was inactivated on chicken breast, beef steak, and catfish fillet surfaces at a UV-C dose of 1 J/cm^2. UV-C treatment prior to freezing of the foods did not increase the inactivation of *Y. pestis* over freezing alone. These results indicate that routine use of UV-C during food processing would provide workers and consumers some protection against *Y. pestis*.

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1. Introduction

Yersinia species including *Yersinia enterocolitica*, *Yersinia pseudotuberculosis*, and *Yersinia pestis* are closely related species which can cause illness in humans (Paoli and Sommers, 2014). Of those 3, *Y. pestis* is the causative agent of plague and can cause pharyngeal or gastrointestinal infection through the handling or consumption of meat products (Leslie et al., 2010; Albaji et al., 2005; Bin Saeed et al., 2005; Christie et al., 1980). The risk, and the associated morbidity and mortality, of contracting plague through the consumption or handling of foods deliberately contaminated with *Y. pestis* are currently unknown. Because *Y. pestis* is listed as a select agent for both food safety and food defense (Brickner et al., 2003; Khan et al., 2000; Kauffman et al., 1997; WHO, 1970) it would therefore be prudent to evaluate the efficacy of nonthermal food processing intervention technologies to inactivate *Y. pestis* in food products.

Recent studies have investigated the ability of *Y. pestis* to grow in foods, including growth at refrigeration temperature. Bhaduri and Phillips (2013) found that *Y. pestis* KIM5 did not grow in refrigerated ground pork (4 °C), but was able to grow at the mild refrigeration abuse temperatures of 10 and 15 °C at ca. 0.05 and 0.16 $\log_{10}$ CFU/h, respectively. *Y. pestis* KIM5 readily grew at 20, 25 and 30 °C at 0.26, 0.30 and 0.77 $\log_{10}$ CFU/h. Sommers and Cooke (2009) found that *Y. pestis* was capable of growth on frankfurters at 10 °C, while Gurtler et al. (2010) found *Y. pestis* capable of growth in refrigerated liquid egg. Torosian et al. (2009) found that *Y. pestis* was capable of growth in Brain Heart Infusion Broth at 4 °C.

Other studies investigated the use of both thermal and nonthermal intervention technologies to inactivate *Y. pestis* in foods. Sommers and Niemira (2007) investigated the use of gamma radiation at both refrigeration (D_{10} 0.19 at 10 °C) and frozen temperatures (D_{10} 0.37 kGy at −20 °C) and developed a predictive equation to describe the temperature dependent radiation inactivation kinetics of *Y. pestis* in ground meat. Sommers and Cooke (2009) found the gamma radiation D_{10} of a multi-isolate cocktail of *Y. pestis* inoculated onto phosphate buffer and frankfurters to be 0.23 and 0.31 kGy, respectively. Porto-Fett et al. (2009) determined the thermal D_{10} value of *Y. pestis* KIM5 suspended in 75% lean

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ground beef at 48.9–60 °C to range from 193 to 0.56 min, respectively, with a z-value of 4.57 °C.

Ultraviolet light (UV-C, 254 nm), a U.S. Food and Drug Administration (FDA) approved food safety intervention technology, can be used for decontamination of food and food contact surfaces (CFR, 2005). UV-C exerts its bacteriocidal effect primarily through the formation of DNA adducts including cyclobutane pyrimidine dimers and 6–4 photoproducts, either killing the bacteria or rendering them unable to reproduce, and to some extent through oxidation of proteins in the bacterial cell (Reardon and Sancar, 2005; Krisko and Radman, 2010). The technology has been increasingly adopted by the health care industry for decontamination of surfaces in medical facilities and in recent years the food processing industry has expressed significant interest in using it for surface decontamination of foods and food contact surfaces in the food processing environment (Salvage, 2014; Rutala et al., 2010; Koutchma, 2008; Koutchma et al., 2008). UV-C is of particular appeal to the medical and food processing industries because it is a green and sustainable technology which does not require the use of water or chemicals to exert its bacterial effects.

While a great deal of information is available on the use of ultraviolet light (UV-C) to inactivate common foodborne pathogens (Sommers et al., 2010; Sumner et al., 1996; Stermer et al., 1987), relatively little information is available on the inactivation of *Y. pestis* on foods with ultraviolet light. The purpose of this study was to determine the ability of *Y. pestis* to survive treatment with UV-C on agar plates, food contact surfaces, and foods using a commercial UV-C conveyor.

2. Materials and methods

2.1. Food products

Catfish fillets (2–3 oz) were obtained from the USDA-ARS Catfish Genetics Laboratory in Stoneville, Mississippi. Beef steaks (3–4 oz) and boneless skinless chicken breast fillets (3–4 oz) were obtained fresh from a local butcher. The meat, poultry, and ready-to-eat meat products were gamma irradiated to an absorbed dose of 5 kGy at 4 °C to inactivate background microflora, which was reduced to less than to <0.1 CFU/g as determined by plating on Brain Heart Infusion agar (BD-Difco, Sparks, MD). Exudate (drip/purge) was obtained from the irradiated products by pouring excess liquid into sterile test tubes. Food was stored at 0–4 °C until ready for use, while exudates were stored at −20 °C.

2.2. Avirulent Yersinia pestis isolates

Four avirulent *Y. pestis* strains (KUMA, Yokohama, KIM5, and CO99) were obtained from Dr. Susan Straley (Univ. Kentucky) and Dr. Robert Brubaker (Mich. State Univ.) through Dr. George Paoli at USDA's Eastern Regional Research Center (Wyndmoor, PA). The *Y. pestis* strains were propagated on Brain Heart Infusion Agar (BHIA) (BD-Difco, Sparks, MD) at 30 °C for approximately 3 days and maintained at 0–4 °C until ready for use.

2.3. Propagation and inoculation

Each *Y. pestis* strain was cultured independently in 30 ml BHI Broth (Brain Heart Infusion, BD-Difco, Sparks, MD) in a sterile 50-mL conical tube at 30 °C (150 rpm) for 36 h, to a density of approximately 10^8 CFU/ml, using a New Brunswick Model G24 Incubator (Edison, NJ). The cultures were then sedimented by centrifugation (1200 × *g*) using a Fisher Scientific Marathon 21000R Centrifuge (Needham Heights, MA). The cell pellets were then resuspended as a cocktail in 30 mL Butterfield's Phosphate Buffer (BPB) (Applied Research Institute, Newtown, CT).

One hundred μL (0.1 mL) of the *Y. pestis* undiluted cocktail was plated onto duplicate BHIA plates which were then allowed to dry for approximately 5 min. The agar plates were then exposed to 0.5 J/cm^2 UV-C using the UV-C conveyor as described below.

For experiments using exudates on high density polypropylene (HDPP) and polyethylene (HDPE) and stainless steel coupons (bead blasted and electropolished) 0.1 mL of *Y. pestis* cocktail was pipetted into 0.9 mL of the sterile food exudates, mixed by vortexing for ca. 10 s, and 0.1 mL transferred to the chilled (4 °C) food contact surfaces and exposed to UV-C as described below. Prior to experimentation stainless steel coupons were sterilized by autoclaving while HDPP and HDPE were cleaned with 70% ethanol and then subjected to 10 J/cm^2 UV-C.

Chicken, fish, and beef pieces were inoculated on one side of the surface with 1.0 mL (ca. 10^6 CFU/ml), which was then spread on the food surface (3 × 3 cm) using a sterile inoculation loop. The inoculated pieces were allowed to sit in a refrigerator (4 °C) for ca. 30 min, and then passed through the conveyor to obtain the required UV-C doses (0.25, 0.5, 1.0 and 2.0 J/cm^2).

Following exposure to UV-C, food samples were placed in sterile polynylon bags (Uline, Inc., Philadelphia, PA) containing 100 ml BPB and rinsed manually by massaging for ca. 30 s. The solution was then serially diluted in 9 ml BPB and two 0.1 mL aliquots per dilution were spread on BHI agar plates (BD-Difco, Sparks, MD). The plates were then incubated for 3 days at 30 °C prior to enumeration of bacterial colonies. Each experiment was conducted independently a minimum of 3 times (n = 3).

2.4. Exposure to ultraviolet light (UV-C)

A commercial food-grade UV-C conveyor (Reyco Systems, Inc., Meridan, ID) was used to treat exudates inoculated with avirulent *Y. pestis* on surfaces of BHI agar plates, stainless steel coupons, HDPP and HDPE, and the foods themselves. The UV-C intensity was 5 mW/s/cm^2, and 100 s of exposure provided a total UV-C dose of approximately 0.5 J/cm^2. All experiments were conducted in a BSL-2 laboratory.

The exudates (0.1 mL, ca. 10^7 CFU) were inoculated onto BHI agar plates, stainless steel or polypropylene coupon surfaces were run through the conveyor either once, twice or four times to obtain exposures of 0.5 J/cm^2, 1.0 J/cm^2 or 2.0 J/cm^2, respectively. Beef steaks, chicken breast, and catfish fillets were passed through the conveyor up to 4 times to obtain the maximum UV-C dose of 2.0 J/cm^2.

UV-C intensity was monitored using a calibrated UVX Radiometer (UVP Inc., Upland, CA). The temperature of the room was approximately 15 °C during the exposure to UV-C, and the food temperature did not increase to more than 15 °C at the end of the process as measured using an infrared thermometer.

2.5. Freezing

Catfish fillets, chicken breasts, and beef steaks were inoculated and treated with UV-C as described above and then frozen using dry ice. The individual samples were then packaged in polynylon bags (Uline Inc., Philadelphia, PA), sealed using a Multi-Vac 300 packager to ca. 5000 Pa and stored at −20 °C for ca. 30 days. To assess bacterial counts after freezing, the samples were thawed in a refrigerator (ca. 4 °C) overnight.

2.6. Statistical analysis

Log reductions were determined according to Diehl (1995). Each experiment was conducted independently a minimum of 3 times.