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Inactivation of *Francisella tularensis* Utah-112 on Food and Food Contact Surfaces by Ultraviolet Light

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Abstract

Francisella tularensis is the causative agent of tularemia, a plague-like illness that affects animals and humans, and has caused large illness pandemics in the last century. It has also been used as a biological warfare agent, and tularemia can be contracted through consumption of contaminated food and water. In this study the use of a U.S. Food and Drug Administration approved technology, 254 nm ultraviolet light (UV-C), to inactivate *F. tularensis* Utah-112 (a rodent pathogen) on food and food contact surfaces was investigated. The D_{10} value, the UV-C dose needed to inactivate one log of microorganism, was approximately 0.71 mJ/cm^2 on agar plates using a low UV-C intensity of 100μW/cm^2/s. When a commercial UV-C conveyor was used (5 mW/cm^2/s) 0.5 J/cm^2 inactivated >7 log CFU of *F. tularensis* Utah 121 on agar plates. At 0.5 J/cm^2 UV-C inactivated >4 log CFU of Utah-112 in beef, chicken, catfish, frankfurter, and bratwurst exudates inoculated onto stainless steel coupons, and >7 log CFU was eliminated at 1 J/cm^2 UV-C. Similar results were obtained when the exudates were inoculated onto high density polypropylene. Approximately 0.5 log CFU was inactivated on chicken breast, beef steak, and catfish fillets, and approximately 1.9 log CFU on frankfurters and bratwurst at a UV-C dose of 1 J/cm^2. These results indicate routine use of UV-C during food processing would provide workers and consumers some protection against *F. tularensis*.

Keywords: *Francisella tularensis*; Ultraviolet; Food; Food contact; Beef; Sausages; Chicken; Tilapia; Catfish

Introduction

Francisella tularensis is the bacterium responsible for a lethal plague-like illness in humans and animals known as tularemia. It has been responsible for large epidemics in Europe and Asia, and causes epizootic illnesses elsewhere in the world [1]. The utility of *F. tularensis* as a biological weapon was studied extensively by during World War II, and the microorganism may have used in that conflict for that purpose. During the Cold War *F. tularensis* was studied for its potential as a biological weapon [1,2]. Various scenarios have been developed by international health organizations to assess the health and economic impacts of a biological attack using *F. tularensis* [3,4]. *F. tularensis* is now considered a category A biological agent by the Centres for Disease Control and Prevention (CDC) [2].

Tulermia may be contracted through consumption of contaminated food and water and it is also possible to contract the disease by handling infected animals in a food processing environment [5]. Fortunately, the infectious dose required to cause Tulermia through consumption of contaminated foods may be higher than that required to cause infection by inhalation. The LD_{50} for mice given *F. tularensis* by gastric lavage was approximately 10^6 CFU, much greater (>100,000 fold) than for intradermal injection or exposure by aerosol [6,7]. Human volunteers who consumed 10^{10} CFU of *F. tularensis* in vaccine development studies required treatment with antibiotics to prevent imminent death [8]. Studies with primates indicated an oral dose of 10^6-10^7 CFU of *F. tularensis* required intervention with antibiotics to ensure survival [9]. Currently, there is very little information available regarding the utility of food safety intervention technologies to inactivate *F. tularensis* on food and food contact surfaces. The thermal D_{10} (the energy needed to inactivate one log of microorganism) of *F. tularensis* in infant formula and fruit juice has been determined [10]. High pressure (500 mPa, 2 min) inactivated 5 log CFU of *F. tularensis* LVS in milk or orange juice [11]. Ultraviolet light inactivated >7 log CFU of *F. tularensis* in distilled water at a UV-C dose of 4 mJ/cm^2 [12]. 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 [13]. Ultraviolet light exerts its bactericidal 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 [14]. It has also been suggested as a possible intervention technology for mitigation of biological threats [15]. The technology is currently used by a number of industries, including the health care industry for the decontamination of hospital rooms, and is slowly beginning to see increased usage by the food industry [16-20]. Because the oral infectious dose, and LD_{50}, for *F. tularensis* may be relatively high in comparison to exposure by aerosol, food safety intervention technologies which inactivate food borne pathogens may provide some protection against Tulermia for workers and consumers. The purpose of this study was to determine the effectiveness of UV-C for inactivating *F. tularensis* on the surface of solid agar growth medium, in food exudates (also known as drip or purge) placed on food contact surfaces, and on food.

Materials and Methods

Francisella tularensis strains

Francisella tularensis strains LVS, NIH B-38, and Utah-112 were obtained from BEI Resources (Manassas, VA). *F. tularensis* LVS and

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NIH B-38 are attenuated strains which have been used for vaccine development, while Utah-112 is a rodent pathogen. *F. tularensis* was propagated on fluidthioglycolate medium containing 1% agar (FTG, BD Difco, Sparks, MD) at 37°C until ready for use. Liquid cultures for use in experiments were prepared by inoculating single colonies into 10 ml of freshly prepared FTG medium in sterile 50 ml test tubes (Fisher Scientific, USA) and incubating (37°C) in a shaking incubator (150 rpm) (New Brunswick, Inc., Model G24, Edison, NJ) for approximately 24 h.

Food products

Catfish fillets were obtained from the USDA-ARS Catfish Genetics Laboratory in Stoneville, Mississippi. Beef steaks, boneless skinless chicken breast fillets, frankfurters, and bratwurst were obtained from local retailers. The meat, poultry, and ready-to-eat meat products were gamma irradiated, to an absorbed dose of 10 kGy to inactivate background microflora to less than 1 CFU/g. Exudate (drip/purge) was obtained from the irradiated products by pouring excess liquid into sterile test tubes.

Determination of *F. tularensis* D_{10} values on agar plates.

Prior to determination of D_{10} values, media and growth conditions were evaluated for the recovery of *F. tularensis* from agar plate cultures and inoculated foods. Blood-Cystine-Dextrose agar, Mueller-Hinton agar and Fluid Thioglycollate (FTG) medium and incubation in either an aerobic or a 5% CO_2 environment were tested. Stationary phase cultures individually propagated and decimally diluted (0.1 ml culture in 0.9 ml diluent) in Butterfield's Phosphate Buffer (BPB, Applied Research Institute, Newtown, CT, USA) using sterile borosilicate test dilutions were then plated on duplicate FTG agar plates which were then allowed to dry for approximately 30 min. Exposures to UV-C were then carried using the source from a biological safety cabinet (NuAire, Inc., Model No. NU-425-800, Plymouth, MN)) at a UV-C intensity of approximately 100 μW/cm/s, for 10, 20, 30, and 40 s, to obtain UV-C doses of 1, 2, 3, and 4 mJ/cm^2. The FTG plates were then incubated from 2-5 days (37°C) prior to enumeration of colony forming units (CFU). The agar plates for strain Utah-112 could be enumerated after 2-3 days, while strains LVS and NIH B-38 required 4-5 days incubation. The D_{10} values, the UV-C radiation doses needed to inactivate 90% *F. tularensis*, were calculated from the enumeration data. The average bacterial population of the UV-C treated sample (N) was divided by the average population in the untreated control (N_0) to produce a survivor ratio (N/N_0). Radiation D_{10}-values were determined by calculating the reciprocal of the slope of the log (N/N_0) ratios versus the irradiation dose. Each experiment was conducted independently three times (n=3) [21].

Exposure inoculated foods and exudate to ultraviolet light (UV-C)

A commercial food-grade UV-C conveyor (Reyco Systems, Inc. Meridan, Idaho) was used to treat exudates inoculated with *F. tularensis* on surfaces of stainless steel coupons, high density polypropylene (Oneida, Inc, Syracuse, NY), and the foods themselves [22,23]. The UV-C intensity was 5 mW/s/cm^2, and 100 seconds of exposure provided a total UV-C dose of approximately 0.5 J/cm^2. The exudates were inoculated onto stainless steel or polypropylene coupon surfaces and were run through the conveyor either once or twice to obtain exposures of 0.5 J/cm^2 or 1.0 J/cm^2, respectively. Foods 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 measured using a calibrated UVX Radiometer (UVP, Inc, Upland, CA). The conveyor automatically rotated the frankfurters and bratwurst, which are cylindrical, during the exposure period. The temperature of the room was approximately 15°C during the exposure to UV-C, and food temperature did not increase to more than 20°C at the end of the process as measured using an infrared thermometer [24].

UV-C inactivation of *F. tularensis* Utah-112 in exudate (drip)

Exudates (drip/purge) were collected from the packages of irradiated food products. Overnight *F. tularensis* culture was then diluted 1/10 in the exudates and mixed by vortexing for approximately 10 seconds. Following mixing, 0.1 ml of exudate (10^7 CFU/ml) was pipeted onto a stainless steel coupon, which was then given either one or two passes through the UV-C conveyor (0.5 and 1.0 J/cm^2). The stainless steel coupon was then placed in a sterile polynylon bag with 0.9 ml of FTG medium, serially diluted in BPB, and 0.1 ml spread into duplicate FTG agar plates. The plates were allowed to dry for approximately 30 min and then incubated for approximately 3 days at 37°C prior to enumeration of CFU.

UV-C inactivation of *F. tularensis* Utah-112 on foods

Chicken, fish, and beef pieces (approximately 3 oz) were surface inoculated with 0.1 ml (106 CFU/ml) of a dilution of Utah-112 which was then spread on the food surface using a sterile inoculation loop, allowed to sit in a refrigerator (4°C) of for 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. The solution was then serially diluted in 9 ml BPB and two 0.1mL aliquots per dilution were spread on FTG agar plates. The plates were then incubated in for 2-5 days at 37°C prior to enumeration of bacterial colonies (Lab-Line Amb-Hi- Low Chamber, Melrose, IL, USA). Each experiment was conducted independently 3 times (n=3).

Results and Discussion

To date there is a dearth of data regarding the ability of food safety intervention technologies, including UV-C, to inactivate the biothreat agent *F. tularensis* on food and food contact surfaces. Rose and O'Connell [12] found that a UV-C dose of 4 mJ/cm^2 inactivated *F. tularensis* LVS and NY98 by 6.6 and 8.7 log CFU, respectively, when suspended in water, indicating a D_{10} of approximately 0.5-1 mJ/cm^2. In the current study, the ability of UV-C to inactivate *F. tularensis* on agar medium, food exudates on a food contact surfaces (stainless steel), and food was investigated. The study, in addition to determining inactivation kinetics in a typical laboratory setting, also investigated inactivation of *F. tularensis* using commercial ultraviolet light food decontamination equipment.

Initially the D_{10} value, the UV-C dose needed to inactivate one log of microorganism, of three *F. tularensis* strains Utah-112, LVS, and NIH B-38 were determined when inoculated onto thioglycollate agar plates. The D_{10} values for those three strains are listed in table 1, with Utah-112 being the most resistant to UV-C with a D_{10} of 0.71 J/cm^2. These results are at the low end of the range of D_{10} reported for food borne pathogens and bacteria of 1.5-8 J/cm^2 as reported in other studies [25]. When the three *F. tularensis* strains were inoculated on agar plates and then run through the commercial UV-C conveyor (0.5 J/cm^2, 5 mW/cm^2/s) no colonies were recovered (>7 log CFU reduction). We observed that D_{10} for Utah-112 could be determined in 2- 3 days while the D_{10} for LVS and NIH B-38 required incubation for 4-5 days

for colony growth, enumeration, and D_{10} determination. In addition, Utah-112 gave more consistent results than LVS and NIB B 3-8 when recovering *F. tularensis* from actual food matrices. Therefore, Utah-112 was used for inactivation studies in food exudates.

Inactivation of pathogens on food and food contact surfaces is important as workers could come in contact with foodborne pathogens such as *F. tularensis* during the handling of foods or the cleaning and sanitation of equipment [5]. Inactivation of the bacteria prior to spraying and washing of equipment, as part of the equipment sanitation process, could mitigate the risk to plant workers from inhalation of *F. tularensis* aerosols. When *F. tularensis* Utah-112 was inoculated in beef, chicken, catfish, tilapia, frankfurter, or bratwurst exudates, which was then placed on the surface of stainless steel coupons, a UV-C dose of 0.5 J/cm^2 inactivated 4.56-5.10 log CFU of *F. tularensis* Utah-112 (Figure 1A). Similar results were obtained when the microorganism was inoculated onto high density polypropylene (Figure 1B). The pathogen was reduced >7 log CFU (essentially eliminated) at a UV-C dose of 1.0 J/cm^2.

The inactivation of *F. tularensis* Utah-112 inoculated onto actual food products and exposed to UV-C (0.25-2.0 J/cm^2) (Figure 2). In each case the inactivation obtained was equal to or superior to those of other food borne pathogens including *Escherichia coli* O157:H7, *Listeria monocytognes*, *Salmonella spp.*, *Staphylococcus aureus,* and *Yersinia pestis* using similar commercial grade equipment when inoculated products were treated with UV-C doses ranging 0.5 to 2.0 [23-25]. Similar results, although more inconsistent, were obtained with *F. tularensis* LVS and NIH B-38 (not reported). Although the dose exceeded that typically needed to inactivate *F. tularensis* on the surface of agar plates, higher intensities are typically required to inactivate microorganisms in the presence of particulate matter, UV absorbing organic materials, biofilms, or within tissue [26]. The tailing effect observed on actual meat and fish products has been described in previous studies, and can be attributed to shielding effects due to topological differences in food surface and food surface porosity [26,27].

Strain	D10 (mJ/cm2)	R2
Utah-112	0.71a	0.96
LVS	0.62b	0.95
NIH B-38	0.64b	0.94
Mean (SEM)	0.66(0.03)	N/A

Table 1: Ultraviolet light inactivation of *Francisella tularensis* on thioglycollate agar plates. Each experiment was conducted independently 3 times. D_{10} values with the same superscript letters are statistically similar as determined by student's t-test ($p<0.05$). Standard error of the mean (SEM) is shown in parenthesis.

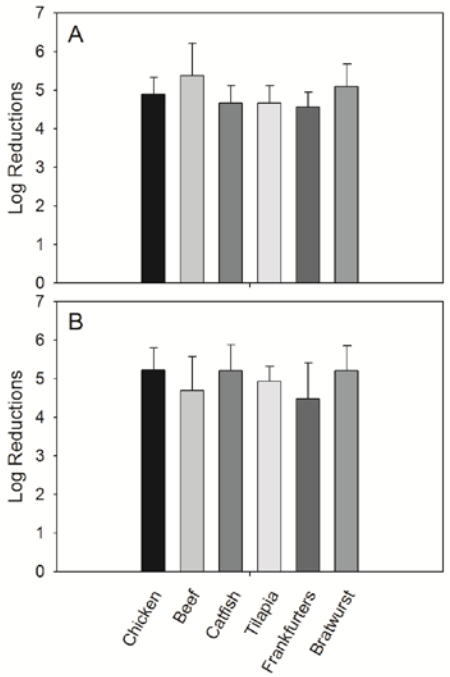

Figure 1: Inactivation of *F. tularensis* Utah-112 in food exudates inoculated onto: **A)** stainless steel; and **B)** polypropylene by ultraviolet light (UV-C). The UV-C dose was 0.5 J/cm^2. Each experiment was conducted independently 3 times. Standard error of the mean is shown as error bars. There was no difference in the mean log reductions as determined by ANOVA (n=3, $p>0.05$).

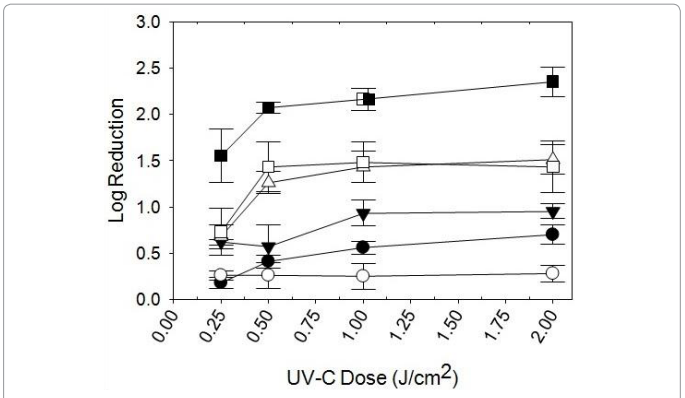

Figure 2: Inactivation of *F. tularensis* Utah-112 on foods by ultraviolet light (UV-C). UV-C doses for beef (o), chicken (●), catfish (▼), tilapia (Δ), bratwurst (□), and frankfurters (■) were 0.25, 0.5, 1.0 and 2.0 J/cm^2. Each experiment was conducted independently 3 times. Standard error of the mean is shown as error bars.

It has been suggested previously that UV-C light technology has potential for use to combat and mitigate acts of bioterrorism [15]. In this study it was determined that the sensitivity to UV-C was below 1 J/cm^2 for *F. tularensis* on agar surfaces, that *F. tularensis* was effectively eliminated from food contact surfaces when suspended on beef, poultry, and fish exudates and that inactivation of *F. tularensis* on actual beef, poultry and fish surfaces was consistent with inactivation for other food borne pathogens. Thus, UV-C light technology could mitigate the risk of *F. tularensis* contamination in a food processing environment.

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