Inactivation of Avirulent *Yersinia pestis* in Butterfield’s Phosphate Buffer and Frankfurters by UVC (254 nm) and Gamma Radiation†

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ABSTRACT

*Yersinia pestis* is the causative agent of plague. Although rare, pharyngeal plague in humans has been associated with consumption or handling of raw or cooked meat products prepared from infected animals. The risks of contracting plague from consumption of deliberately contaminated food are currently unknown. Gamma radiation is a penetrating form of electromagnetic radiation, and UVC radiation is used for decontamination of liquids or food surfaces. Gamma radiation $D_{10}$-values (the radiation dose needed to inactivate 1 log unit pathogen) were 0.23 (+0.01) and 0.31 (+0.03) kGy for avirulent *Y. pestis* inoculated into Butterfield’s phosphate buffer and onto frankfurter surfaces, respectively, at 0°C. A UVC radiation dose of 0.25 J/cm² inactivated avirulent *Y. pestis* suspended in Butterfield’s phosphate buffer. UVC radiation doses of 0.5 to 4.0 J/cm² inactivated 0.97 to 1.20 log units of the *Y. pestis* surface inoculated onto frankfurters. A low gamma radiation dose of 1.6 kGy could provide a 5-log reduction and a UVC radiation dose of 1 to 4 J/cm² would provide a 1-log reduction of *Y. pestis* surface inoculated onto frankfurters. *Y. pestis* was capable of growth on frankfurters during refrigerated storage (10°C). Gamma radiation of frankfurters inhibited the growth of *Y. pestis* during refrigerated storage, and UVC radiation delayed the growth of *Y. pestis*.

Pharyngeal plague can be contracted through the handling or consumption of raw or cooked meat products prepared from animals infected with the pathogen *Yersinia pestis* (1–3, 6). The risk of contracting plague, and the associated morbidity and mortality, 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 (21), the efficacy of nonthermal food processing intervention technologies to inactivate *Y. pestis* in food products should be evaluated.

Gamma radiation and UVC radiation (254 nm) are nonthermal processing technologies that can be used to decontaminate foods or food surfaces. Ionizing radiation can inactivate foodborne pathogens such as *Escherichia coli* O157:H7, *Salmonella*, *Staphylococcus aureus*, *Listeria monocytogenes*, and *Y. enterocolitica* on raw and ready-to-eat meat products (5, 12, 20, 22). A petition to allow irradiation of ready-to-eat foods is currently pending with the U.S. Food and Drug Administration (FDA) (8). UVC is an FDA-approved source of radiation for decontamination of food surfaces (24).

Although much information is available on the use of radiation to inactivate common foodborne pathogens (5, 12, 20, 22), relatively little information is available on the radiation (gamma or UVC) needed to inactivate *Y. pestis*. The purpose of this study was to determine the ability of *Y. pestis* to survive treatment with gamma or UVC radiation when the pathogen was suspended in buffer or was surface inoculated onto frankfurters that contained the commonly used antimicrobials potassium lactate and sodium diacetate (10, 23).

MATERIALS AND METHODS

Frankfurters. Freshly made frankfurters were purchased from a local manufacturer. The frankfurters consisted of beef, pork, water, salt, flavoring, paprika, sodium phosphate, sodium diacetate (0.07%), potassium lactate (1.13%), sodium erythorbate, and sodium nitrate. The antimicrobials sodium diacetate and potassium lactate are commonly used in commercially available frankfurters to inhibit the growth of *L. monocytogenes*. Frankfurters were frozen at −20°C and gamma irradiated at a dose of 10 kGy to eliminate background microflora.

*Y. pestis*. Four avirulent *Y. pestis* strains (KUMA, Yokohama, KIMS, and CO99) were obtained from Dr. Susan Straley (University of Kentucky, Lexington) and Dr. Robert Brubaker (Michigan State University, East Lansing) through Dr. George Paoli at the U.S. Department of Agriculture Eastern Regional Research Center (Wyndmoor, PA). The *Y. pestis* strains were propagated on brain heart infusion (BHI) agar (Difco, Becton Dickinson, Sparks, MD) at 30°C for approximately 3 days and maintained at 0 to 4°C until ready for use. Sommers and Novak (18) previously determined that there is no virulence factor–dependent difference in the radiation resistance between virulent and avirulent forms of the closely related bacterium *Yersinia enterocolitica* when sus-
TABLE 1. Gamma radiation inactivation of *Yersinia pestis* suspended in Butterfield’s phosphate buffer or surface inoculated onto frankfurters

<table>
<thead>
<tr>
<th>Culture type</th>
<th>0 kGy</th>
<th>0.25 kGy</th>
<th>0.50 kGy</th>
<th>0.75 kGy</th>
<th>1.0 kGy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>0.00 (±0.00)</td>
<td>1.01 (±0.05)</td>
<td>2.07 (±0.03)</td>
<td>3.10 (±0.02)</td>
<td>4.21 (±0.04)</td>
</tr>
<tr>
<td>Frankfurter</td>
<td>0.00 (±0.00)</td>
<td>0.99 (±0.13)</td>
<td>1.76 (±0.18)</td>
<td>2.33 (±0.22)</td>
<td>3.38 (±0.03)</td>
</tr>
</tbody>
</table>

*Log reductions are the mean of three independent experiments, with the standard error of the mean shown in parentheses.*

TABLE 2. *UVC light inactivation of Yersinia pestis* suspended in Butterfield’s phosphate buffer or surface inoculated onto frankfurters

<table>
<thead>
<tr>
<th>Culture type</th>
<th>0 J/cm²</th>
<th>0.13 J/cm²</th>
<th>0.25 J/cm²</th>
<th>0.5 J/cm²</th>
<th>1.0 J/cm²</th>
<th>2.0 J/cm²</th>
<th>4.0 J/cm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>0.00 (±0.00)</td>
<td>3.81 (±0.55)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Frankfurter</td>
<td>0.00 (±0.00)</td>
<td>0.97 (±0.08)</td>
<td>1.01 (±0.07)</td>
<td>1.10 (±0.06)</td>
<td>1.20 (±0.41)</td>
<td>1.30 (±0.61)</td>
<td>1.40 (±0.81)</td>
</tr>
</tbody>
</table>

*Log reductions are the mean of three independent experiments, with the standard error of the mean shown in parentheses. ND, not detected.*
Scanning electron microscopy. Frankfurters were inoculated and UVC irradiated as described previously. Thin (1 mm) 1-cm slices of the surfaces of frankfurter samples were excised with a stainless steel razor blade and immersed in ~20 ml of a 2.5% glutaraldehyde–0.1 M imidazole buffer solution (pH 7.2). The samples were then stored for a few days in sealed vials. For sample processing, slices were washed in imidazole buffer and then dehydrated by exchange with 20-ml volumes of graded ethanol solutions (50, 80, and 100%), with two changes at each concentration. Samples were then critical point dried with liquid CO₂, and dry slices were glued with Duco cement (ITW Performance Polymers, Riviera, FL) and colloidal silver adhesive (Electron Microscopy Sciences, Hatfield, PA) to specimen stubs. The mounted samples were sputter coated with gold, and digital images were captured with a model Quanta 200 FEG scanning electron microscope (FEI Co., Inc., Hillsboro, OR) operated in the high-vacuum secondary electron imaging mode (10 kV).

Confocal laser scanning fluorescence microscopy. Frankfurters were inoculated and UVC irradiated as described previously. Thin (2 to 3 mm) slices of the surface of frankfurter sam-

Statistical analysis. Each experiment was conducted independently three times. Statistical analysis functions of MS Excel (Microsoft Corp., Redmond, WA) were used for routine calculations, descriptive statistics, and analyses of variance.
dye kit L-7012 (Molecular Probes, Eugene, OR) (3 and gamma (triangle) radiation. Controls (closed circle) and samples exposed to UVC (open circle) were immersed in 1 ml of the nucleic acid staining viability dye kit L-7012 (Molecular Probes, Eugene, OR) (3 µl of A + B components per ml of imidazole buffer solution). After 20 min of incubation in the dye solution, concentrated glutaraldehyde was added to a final concentration of 2.5%, samples were transferred into 50 µl of staining solution in a glass-bottom microwell dish (MatTek Corp., Ashland, MA), and surfaces images were captured with a model IRBE optical microscope and a 63× water immersion lens coupled to a TCS-SP confocal fluorescence attachment (Leica Microsystems, Exton, PA). Fluorescence was excited by the 488-nm line of an Argon laser, and emission data were collected simultaneously as stacks of optical sections into two channels, 500 to 540 nm for glutaraldehyde-induced autofluorescence (marking the superficial matrix of the frankfurter and fluorescence from the viability dye, Syto 9, labeling the microbial cells) and 640 to 680 nm for the “nonviable” dye, propidium iodide. Maximum projection images of the two stacks of optical sections in the two channels were overlaid and compared to determine the abundance of viable and nonviable cells on the convoluted surfaces of treated and nontreated samples.

**RESULTS AND DISCUSSION**

Gamma radiation, emitted as photons from cobalt-60 or cesium-137, is a penetrating form of electromagnetic radiation that can be used to inactivate foodborne pathogens either in or on meat products. Gamma radiation exerts its effects directly by induction of single- and double-strand DNA breaks and indirectly by oxidative DNA damage through the radiolysis of water (11, 15). In this study, the mean (± standard error of the mean) D<sub>10</sub>-values (the radiation dose needed to inactivate 90% of a foodborne pathogen) were 0.23 (±0.01) kGy (R<sup>2</sup> = 0.99) for Y. pestis suspended in buffer and 0.31 (±0.01) kGy (R<sup>2</sup> = 0.96) for Y. pestis inoculated onto frankfurter surfaces (Table 1). Gamma radiation D<sub>10</sub>-values for foodborne pathogens are typically lower in aqueous solutions because of greater exposure to the radiolysis products of water as opposed to suspension in meat products, where other organic molecules interact with free radicals (11, 15).

In previous studies, the gamma radiation D<sub>10</sub>-values for the common foodborne pathogens Salmonella, L. monocytogenes, S. aureus, E. coli O157:H7, and Y. enterocolitica were 0.61, 0.54, 0.47, 0.36, and 0.15 kGy, respectively, when inoculated onto complex ready-to-eat foods, including frankfurters (19). The D<sub>10</sub>-values for Salmonella, L. monocytogenes, S. aureus, and E. coli O157:H7 suspended in refrigerated (4°C) raw pork were 0.51, 0.48, 0.43, and 0.30 kGy, respectively (22). The D<sub>10</sub>-value of Y. enterocolitica suspended in raw ground pork was 0.19 to 0.23 kGy (11, 17, 18). Sommers and Niemira (16) found that the gamma radiation D<sub>10</sub>-value of Y. pestis suspended in refrigerated (0°C) raw ground pork was 0.27 kGy. Therefore, Y. pestis is one of the more gamma-radiation-sensitive foodborne pathogens and could easily be inactivated on meat and ready-to-eat meat products by ionizing radiation.

UVC radiation is a nonpenetrating form of electromagnetic radiation that is used to inactivate microorganisms in solutions and on food surfaces (5, 9, 20, 25). It exerts its effects through creation of cyclobutane pyrimidine dimers and 6-4 photoproducts in chromosomes that interfere with the DNA replication process (7). There is relatively little information on the resistances of common foodborne pathogens on the surfaces of raw meat and ready-to-eat meat products exposed to UVC radiation; however, one researcher listed four pathogens in order of relative resistance: L. monocytogenes > S. aureus > Salmonella > E. coli (5). Y. pestis was inactivated in BPB at a UVC dose of 0.25 J/cm<sup>2</sup> (Table 2). When Y. pestis was surface inoculated into frankfurters, UVC doses of 0.5 to 4 J/cm<sup>2</sup> resulted in 0.97 to 1.2-log reductions (greater than 90% of the pathogen). In a recent study using the same methodology and UVC irradiation apparatus, a UVC radiation dose of 4 J/cm<sup>2</sup> inactivated approximately 2.0 log CFU of L. monocytogenes on frankfurter surfaces (13). Although the surface of the frankfurters used in this study were relatively smooth in comparison to other ready-to-eat meat products such as turkey ham (13), scanning electron microscopy revealed many places where the bacterium could be shielded from UVC radiation (Fig. 1). The survival of the bacterium on frankfurter surfaces, as examined with a confocal laser scanning fluorescence microscope on samples stained with live-dead stains, is shown in Figure 2.

When Y. pestis was inoculated onto frankfurters and growth was assessed during an 8-week storage period (at 10°C), Y. pestis proliferated modestly (Fig. 3). UVC radiation delayed the growth of the pathogen for approximately 4 weeks, but gamma radiation completely inhibited growth of the survivors for 8 weeks.

Neither gamma nor UVC radiation had a negative impact on the quality (color and texture) of frankfurters or bologna that contained potassium lactate or sodium diacetate (13, 14). Y. pestis is very sensitive to gamma radiation in comparison to other common vegetative foodborne pathogens and could easily be inactivated in either aqueous solution or on frankfurters. Y. pestis was very sensitive to UVC radiation when suspended in BPB but was more resistant to UVC radiation than was L. monocytogenes when inoculated onto the surface of frankfurters.

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REFERENCES