Radiation Sensitivity of *Listeria monocytogenes* in Phosphate Buffer, Trypticase Soy Broth, and Poultry Feed

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ABSTRACT

Radiobiological studies were carried out with three strains of *Listeria monocytogenes*. Resistance characteristics were determined under a variety of conditions. For one strain (CFPDC), the $D_{10}$ values were 0.18, 0.21, and 0.44 kGy when subjected to gamma irradiation in phosphate buffer ($pH$ 7.0), trypticase soy broth containing yeast extract (TSB-YE), and in poultry feed, respectively. For this strain, $D_{10}$ values in poultry feed determined by using $^{60}$Co gamma rays or 10 MeV electrons as radiation sources were almost identical. $D_{10}$ values for gamma irradiation of TSB-YE cultures were 0.21, 0.25, and 0.46 kGy for CFPDC, Scott A, and 81-861 strains, respectively.

The bacterium *Listeria monocytogenes* is a motile, Gram-positive, catalase-positive, psychrotrophic cocco-bacillus that can cause serious diseases in humans (1,16) and animals (9). This microorganism is widely distributed in nature and has been isolated from soil, sewage, animal feed, water, and vegetation (1,28,29). Since 1980, *Listeria* has gained increasing recognition as a foodborne pathogen (3,14,15,22). In animals, many cases of listeriosis have been attributed to contaminated silage (8,10). *L. monocytogenes* has been isolated from fresh meats and poultry and surveys have revealed a high incidence of contamination and spoilage of foods (11) (19), but there is limited published information on the radiation sensitivity of *L. monocytogenes*. Such information, for various strains and a range of conditions likely to be encountered in practice, is essential for the development of radiation processing as an effective means of controlling this feed- and foodborne pathogen.

The objective of the present study was to characterize the radiation sensitivity of *L. monocytogenes* with respect to different strains, different suspension media, and gamma and electron-beam radiation.

MATERIALS AND METHODS

Preparation of test samples

*Listeria monocytogenes*, strain CFPDC, was obtained from the Canadian Food Products Development Centre, Portage La Prairie, Manitoba, Canada (courtesy of W. D. Sprung). This strain was originally isolated from food by Dr. J. M. Farber, Bureau of Microbial Hazards, Food and Drug Directorate, Health and Welfare Canada, Ottawa, ON, Canada. The strains Scott A and 81-861 were kindly provided by Dr. J. M. Farber. Cultures were maintained on Difco trypticase soy agar containing 0.6% Difco yeast extract (TSA-YE, Difco, Detroit, MI) by incubation for 24 h at 35°C, followed by storage at 4°C. When required, the culture was streaked onto a TSA-YE plate and incubated for 24 h at 35°C. Colonies were transferred to 25-ml volumes of Difco sterile trypticase soy broth containing 0.6% yeast extract (TSB-YE) and allowed to grow for 24 h at 35°C. Under these conditions, culture densities varied from 2.4 x 10^9 to 3.7 x 10^9 colony forming units (CFU)/ml after 24-h incubation. To make cell suspensions in phosphate buffer, the cells were removed from 25 ml of the 24-h broth culture by centrifugation at 9000 x g for 10 min at 0-2°C. The pellet was suspended in 25 ml of cold sterile buffer (0.067 M NaHPO$_4$, pH 7.0), the centrifugation was repeated, and the pellet was resuspended in 25 ml of cold buffer.
Poultry feed (complete starter ration) was purchased from a local supplier. The feed was powdered in a Waring blender and the powder sterilized by exposure to 25 kGy of gamma irradiation prior to inoculation. To inoculate the feed, 30-μl portions of the 24-h TSB-YE culture were pipetted into 16 x 125 mm sterile screw-cap glass test tubes containing 0.5 g of sterile feed powder, mixed vigorously with a vortex mixer, and kept at 4°C in a refrigerator for 3 h before use.

Irradiation protocols

Suspensions of the organism in broth or phosphate buffer were dispensed in 0.5 ml volumes into sterile screw-cap test tubes. Up to eight tubes were supported in an aluminum disc assembly with holes at the circumference to accommodate the tubes. The assembly was packed in a 2-l beaker of crushed ice and irradiated in a Gammacell 220 (Atomic Energy of Canada Limited). The gamma ray dose rate was 12.2 kGy/h, and the temperature inside the test tubes varied from 0 to 0.5°C during irradiation, as determined by a thermocouple inserted inside a tube containing 0.5 ml of phosphate buffer. A similar procedure was followed for gamma irradiation of 0.5-g portions of the inoculated feed powder.

To irradiate the inoculated poultry feed powder with high-energy electron beam, the test tubes containing the samples were laid horizontally on a plastic tray containing crushed ice. They were covered with a thin layer of crushed ice. The irradiation was carried out by using an I-10/1 linear accelerator located at Whiteshell Research in Pinawa, Manitoba, Canada. This machine produces 10-MeV electrons, with a nominal total beam power of 1 kW. Dose rate at the sample position was approximately 1 kGy/s.

Absorbed radiation doses were determined by using radiographic dye films (GaF) enclosed in test tubes and irradiated with the sample tubes. The absorbance of the irradiated films was measured at 600 nm and the absorbed dose calculated from a calibration curve.

Assay of viable cells and determination of radiation sensitivity

To recover the surviving L. monocytogenes cells, suspensions of the organism in broth or phosphate buffer were serially diluted with 0.1% Difco peptone, and 0.1-ml portions of an appropriate dilution were plated in duplicate on TSA-YE plates. After 40-48 h of incubation at 35°C, plate counts were recorded. Plates with counts in the range of 30-300 colonies were used for analysis. Four replicate samples were irradiated at each dose level, and duplicate plate counts were obtained for each dilution of each sample. Survival curves were constructed by plotting the measured CFU/ml against radiation dose on a semi-log graph. Curves were fitted by linear regression. Radiation sensitivity was expressed as D10 values. A D10 value is defined as the dose required to inactivate a given population to 10% of its initial value; it is determined from the slope of the straight-line portion of the survival curve.

RESULTS AND DISCUSSION

Figure 1 shows the gamma radiation survival curves for L. monocytogenes CFPDC in the three treatment media. All the curves are exponential in form, with no evidence of a shoulder region. The organism was most sensitive when in phosphate buffer, appeared somewhat more resistant (although not statistically significant) in TSB-YE, and was most resistant in the feed powder. The D10 values calculated from the survival curves for the organism irradiated in the buffer, broth medium, and powdered feed were 0.18, 0.21, and 0.44 kGy, respectively. The higher radiation resistance of the organism in the feed compared to the other two media is attributable to differences in media composition, particularly the lower water content of the feed samples; moisture content of the feed before irradiation was 10.4%. It is well known that radiation sensitivity is higher in aqueous media than in dry form because of actions of free radicals generated from water as a result of irradiation (26).

Comparison of the radiation sensitivity of three strains of L. monocytogenes cultured in TSB-YE and irradiated with 60Co gamma rays is shown in Table 1. The D10 values of 0.21, 0.25, and 0.46 kGy for strains CFPDC, Scott A, and 81-861 indicated that significant interstrain variations in radiation sensitivity exist.

Figure 2 compares the sensitivity of L. monocytogenes CFPDC in poultry feed powder to gamma rays from 60Co and to 10-MeV electrons. Although the data points from the electron beam study were scattered around the regression line, the correlation coefficient was high (0.938). A D10 value of 0.47 kGy was calculated from the electron-beam survival curve, which was close to 0.44 kGy calculated from the survival curve based on the gamma ray study. This indicates that, although there are fundamental differences between gamma ray and high-energy electron beams, their lethal effects on microorganism are similar.

The D10 value of 0.44 kGy for L. monocytogenes CFPDC in poultry feed found in this study is quite similar to the values of 0.36, 0.51, and 0.59 kGy found for Salmonella pullorum in fish-, meat-, and blood-meal respectively (6).

The D10 values for L. monocytogenes CFPDC in the buffer and broth media found in this study are comparable to values reported in unspecified broth media for some other vegetative bacteria, including Proteus vulgaris (0.10 kGy), Escherichia coli (0.21 kGy), Pseudomonas fluorescens (0.05), Salmonella enteritidis (0.25 kGy), S. pullorum (0.25 kGy), and Yersinia enterocolitica (0.10 kGy) (27). However, there are some vegetative bacteria that are much more radiation resistant than those listed above (11,27).
The range of $D_{10}$ values reported here is consistent with the work of Patterson (20) who reported $D_{10}$ values of 0.417 to 0.553 kGy for four strains of *L. monocytogenes* irradiated on poultry meat. Similarly, Huhtanen et al. (13) reported $D_{10}$ values of 0.22 to 0.49 kGy for seven strains of *Listeria* irradiated in BNT medium and a range of 0.20 to 1.03 kGy for irradiation in chicken meat. Hashisaka et al. (12) reported $D_{10}$ values of 1.4 and 2.0 kGy for *L. monocytogenes* inoculated into Mozzarella cheese and ice cream, respectively, and irradiated at -78°C. These latter values are not comparable to those reported here, by Patterson (20) or by Huhtanen (13), since the temperature during irradiation differed greatly. Freezing is well known to provide a large protective effect against radiation damage (26).

The data presented indicate that *L. monocytogenes* in various suspending materials, differing widely in chemical composition, can readily be killed by irradiation. Gamma rays and high-energy electrons are equally effective. The measured $D_{10}$ values are consistent with those reported by other workers and indicate that a relatively modest pasteurizing dose of 5 kGy would reduce the population level of viable *L. monocytogenes* by 5- to 10-log cycles in a low-moisture material such as poultry feed. The reduction in viable numbers of *L. monocytogenes* in high-moisture materials would be even greater. Radiation processing readily lends itself to the treatment of bulk commodities. Thus, radiation pasteurization could be an effective method of eliminating this common pathogen from critical components of our food and feed supply.

## REFERENCES


## TABLE 1. Radiation $D_{10}$ values for three strains of *Listeria monocytogenes* suspended in TSB-YE and irradiated with $^{60}$Co gamma rays.

<table>
<thead>
<tr>
<th>Strain</th>
<th>$D_{10}$ (kGy)</th>
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<tbody>
<tr>
<td>CFPDC</td>
<td>0.21</td>
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<tr>
<td>Scott A</td>
<td>0.25</td>
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<td>81-861</td>
<td>0.46</td>
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