

Gamma Radiation Sensitivity of *Listeria monocytogenes*

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ABSTRACT

Seven strains of *Listeria monocytogenes* were irradiated in culture media or in mechanically deboned chicken meat. The survivor plots were quadratic curves when cultures were in the log phase of growth or when they were irradiated in chicken meat; cultures in the senescent phase of growth showed linear responses to irradiation. Cultures from cells surviving an irradiation dose of 1.5 kGy were no more radiation resistant than those which had had no previous exposure to irradiation. Cultures centrifuged and resuspended in water were more sensitive to radiation than those resuspended in solutions containing organic materials. These studies indicated that a dose of 2 kGy was sufficient to destroy 1×10^4 cells of *L. monocytogenes*.

The small gram positive rod named *Listeria* (18), the causative agent of an epizoonosis in laboratory animals, was first described in 1926 by Murray et al. (12). It since has been shown to cause infection in a number of animals as well as in humans. The organism can be isolated from infected animals but also has been isolated from asymptomatic cattle, goats, and sheep; from these the organism may be shed into the milk. Such may have been the case in a recent outbreak involving newborn infants whose mothers had consumed a soft cheese made from raw or improperly processed cows milk (10). *L. monocytogenes* has been shown to be capable of growing readily in milk (13) but it has also been isolated from vegetation in cornfields or other cultivated fields, from feces of deer and birds, forest soils (16,17), and oat silage (8). One epidemic of listeriosis was traced to cole slaw made from cabbage fertilized with the manure from infected sheep (14).

The organism is apparently destroyed by normal pasteurization procedures (2) although a recent report (7) indicated that milk, apparently properly pasteurized, was the vehicle for the infection in an outbreak in Massachusetts. Another report (6) showed that *L. monocytogenes* might survive pasteurization if the bacteria were inside leukocytes. The significance of these related reports was discussed by Donnelly (5).

The character and composition of most raw agricultural products would be adversely affected by heat pasteurization; a possible alternative procedure for destruction of

harmful organisms in such products, with minimal effect on their physical properties, would be to irradiate either with ultraviolet rays as reported by Yousef and Marth (19), or by low dose gamma radiation which has recently been approved for certain agricultural products (4). The objective of the present study was to determine the feasibility of gamma radiation for eliminating the organism from meat and poultry.

MATERIALS AND METHODS

Culture media

The BNT medium was a mixture of 0.4% nutrient broth (Difco) and 1.5% trypticase soy broth with glucose (BBL). The medium was buffered at a pH of 6.4-6.6 with 0.026 M KH_2PO_4 and 0.028 M $\text{NaH}_2\text{PO}_4 \cdot 7 \text{H}_2\text{O}$. BT medium was similarly buffered and consisted of trypticase soy broth with glucose.

Chicken meat

Chicken meat was purchased from a local manufacturer of poultry products and consisted of homogenized, mechanically deboned chicken meat (MDCM) without additives. The meat was 90% ribs and 10% backs with a proximate composition of 63.1% H_2O , 25.7% fat, and 11.4% protein; it was radiation sterilized at 42 kGy in aluminum cans (208 x 107) of 70 g capacity and was kept frozen at a temperature of -10°C . When needed, cans of meat were thawed at room temperature overnight.

Cultures

The organisms used in these studies were seven strains of *L. monocytogenes* (V7, RmI, RmII, Murray B, Scott A, V97, and ATCC 7644). All cultures were incubated at 35°C . Stock cultures were maintained on plate count agar (Difco) slants; transfers were made to tubes of BNT medium which were used to inoculate the experimental media. Cultures in the log phase of growth were prepared by inoculating pre-warmed tubes of BNT with 1% of an 18 h culture and incubating for 1.5, 2.5, or 5.0 h.

Irradiation

A ^{137}Cs source providing a dose rate of 0.125 kGy/min was used for the irradiation. The cultures grown in the organic media were placed in 2 ml quantities into 100 x 13 mm glass screw cap tubes with caps tightly secured. The MDCM in 5 g amounts was placed into 40 ml sterile centrifuge tubes; 0.1 ml of culture was added and mixed in with a sterile wood applicator stick. All cultures in media or in MDCM were kept at a temperature of $2-4^\circ\text{C}$ prior to and during irradiation. The gas phase of liquid nitrogen was used for maintaining temperature during irradiation.

Enumeration of cultures

Following irradiation the cultures were diluted with sterile water at a temperature of 4-7°C. The MDCM samples in the centrifuge tubes were diluted with 20 ml water and were vigorously mixed with caps secured. A spiral plater (Spiral Systems, Inc.) was used to determine cell populations in the culture media. For the cultures irradiated in MDCM a standard pour plating method was employed. Plate count agar (Difco) was used with both methods of plating; incubation of the plates was for 2 d at 35°C. Plating was done in duplicate and in most cases plates from two dilutions were enumerated. Irradiation D values were calculated by dividing the applied dose (in kGy) by the difference between the counts (\log_{10}) of the doses. For example the D value for the first irradiation interval (0-0.50 kGy) was calculated by dividing 0.50 by the difference between the original counts and the counts after irradiation. The reciprocals of the slopes of linear regressions were also used to calculate D values.

Statistical evaluation

The statistical program SAS/STAT (SAS Institute, Cary, NC) was used to determine the significance of the regressions of the irradiation survival plots of the *Listeria* cultures. The graphics program SIGMAPLOT (Jandel Scientific, Corte Madera, CA) was used to obtain the best fit for the curves. Analysis-of-variance of the linear, quadratic, and cubic functions were evaluated; a significant statistical trend was one where the probability for obtaining an F value greater than that observed was between 0.01-0.05; a highly significant statistical trend was one where the probability was less than 0.01.

RESULTS AND DISCUSSION

The results from irradiating *L. monocytogenes* in BNT medium (Table 1) indicated that survivor curves might not be linear although the deviation from linearity was not statistically significant. Non-linearity was suggested by the differences in D values obtained from the first and the last portions of the curve. The seven strains of *L. monocytogenes* had an average D value of 0.27 kGy when the D values were calculated from the dose interval 0-0.50 kGy, while the average D value from the interval 1.00-2.00 kGy was 0.38 kGy. Calculations from linear regression slopes gave an average D value of 0.33 kGy ($r = 0.996$) for the *L. monocytogenes* strains. The increased radiation resistance at the higher doses was more pronounced and was statistically significant for the cultures irradiated in MDCM (Table 2). The average D value for the 0-0.50 kGy dose interval was 0.27 kGy, the same as that for irradiated cultures in BNT medium, but the D value calculated from the 1.00-2.00 kGy doses was 0.77 kGy. D values calculated from linear regression slopes of the MDCM samples gave an average value of 0.45 kGy ($r = 0.981$). Increased radiation resistance in proteinaceous products was reported by Grecz (9) for spores of *Clostridium botulinum* in pork pea broth and canned chicken. A tailing of irradiation survival curves indicating increased radiation resistance at higher doses was

TABLE 1. Irradiation destruction of *Listeria monocytogenes* in BNT medium¹.

Strain	Log CFU/ml					D value (kGy)		
	Control	0.50 ²	1.0	2.0	0-0.50 ³	1.0-2.0	0-2.0	
V7	9.85	7.89	6.37	3.90	0.26	0.40	0.34	
RmI	10.76	8.54	6.86	3.96	0.22	0.34	0.29	
RmII	9.79	7.71	5.94	2.75	0.24	0.31	0.28	
Murray B	10.42	8.48	7.11	4.46	0.26	0.38	0.34	
Scott A	10.33	8.79	7.13	4.23	0.32	0.34	0.33	
V97	9.88	8.34	7.12	4.72	0.32	0.42	0.39	
ATCC 7644	<u>9.69</u>	<u>7.81</u>	<u>5.89</u>	<u>3.84</u>	<u>0.30</u>	<u>0.49</u>	<u>0.34</u>	
Mean	10.10	8.22	6.63	3.98	0.27	0.35	0.33	
Std dev	0.40	0.42	0.56	0.63	0.04	0.09	0.04	

¹See text for composition of BNT medium.

²Dose in kGy.

³D values calculated by the formula: dose (kGy)/(log surviving population dose a - log surviving population dose b).

TABLE 2. Irradiation destruction of *Listeria monocytogenes* in chicken meat.

Strain	Log CFU/ml					D value (kGy)		
	Control	0.50	1.0	2.0	0-0.50	1.0-2.0	0-2.0	
V7	8.78	7.08	6.64	5.02	0.29	0.62	0.53	
RmI	8.95	7.18	6.10	4.45	0.28	0.61	0.44	
RmII	8.76	7.40	6.26	5.01	0.37	0.80	0.53	
Murray B	8.94	6.48	5.36	4.11	0.20	0.80	0.41	
Scott A	7.42	5.90	5.36	3.67	0.20	0.59	0.42	
V97	8.87	7.08	6.88	4.81	0.28	0.93	0.49	
ATCC 7644	<u>8.50</u>	<u>6.51</u>	<u>4.78</u>	<u>3.81</u>	<u>0.25</u>	<u>1.03</u>	<u>0.43</u>	
Mean	8.60	6.80	5.91	4.41	0.27	0.77	0.46	
Std dev	0.54	0.52	0.76	0.56	0.06	0.17	0.05	

See table 1 for explanation of data.

reported by Anellis et al. (1) for spores of *C. botulinum* irradiated at 30 kGy or higher.

Silverman (15) described three types of radiation survival curves exhibited by bacteria. These were: type 1, a convex curve where the radiation resistance decreases with dose; type 3, a concave curve with increasing resistance at higher doses; and type 2, a linear, exponential response. Our results indicating non-linearity of irradiation survival curves was substantiated by analysis-of-variance of the regression plots of the cultures irradiated in MDCM. Survivor plots of strains V7, RmII, and Murray B had highly significant quadratic trends while those from strains V7, RmI, Murray B, V97, and ATCC 7644 also had highly significant cubic trends. Most of the regression of the survivor plots, however, could be accounted for by a linear fit ($r = .95$ or greater). The curves for the averages of the seven strains in BNT medium and in MDCM are shown in Fig. 1. The best fit for both curves were convex quadratic

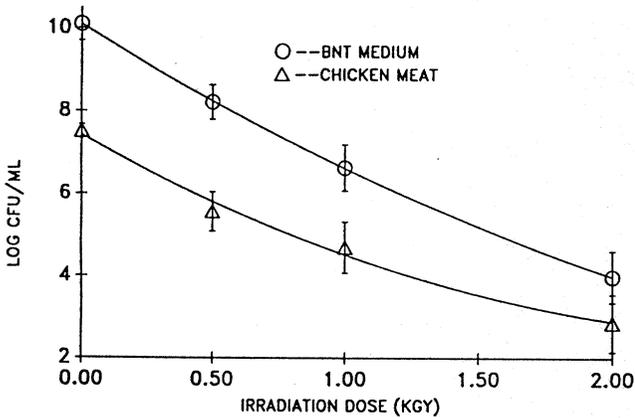


Figure 1. Effect of irradiation on *L. monocytogenes* (survival curves: averages of 7 strains; V7, RmI, RmII, Murray B, Scott A, V97, and ATCC 7644) in either a rich organic medium (BNT) or in mechanically deboned chicken meat.

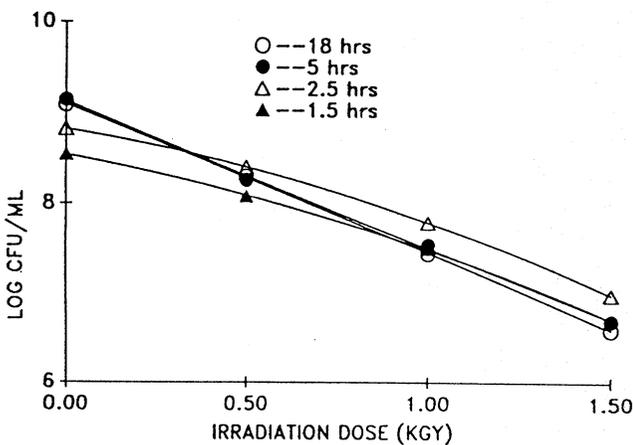


Figure 2. Effect of irradiation on cells in early and late growth stages. Irradiation was in BNT medium with plating in plate count agar incubated at 35°C.

functions. This type of non-linear survivor curve could conceivably be the result of culture inhomogeneity with cells in differing stages of growth exhibiting different resistances to irradiation. This was investigated with the results in Fig. 2. Each point in this graph represents 8 separate determinations of cell numbers. The survivor plots for the fully grown cultures (both the 5 and 18 h cultures showed the same number of cfu/ml) were linear with no significant quadratic or higher component, whereas the rapidly growing cells (which would be expected to be more homogeneous in growth stage) exhibited convex survival curves with highly significant quadratic trends. Thus the cells in the logarithmic phase of growth exhibited concave irradiation destruction curves (type 1) of Silverman while older cultures had type 2 curves. When linear regression lines were fitted to the data, the calculated D values were 0.82 kGy for the 1.5 and 2.5 h cultures ($r = 0.992$), while the value for the 5 and 18 h cultures was 0.61 kGy ($r = 0.999$).

The possibility of cells becoming resistant to radiation was studied by selecting colonies from the highest radiation dose (i.e. from the "tail" of the curves), growing them in BNT medium and subjecting them to further irradiation. The resulting cultures were no more radiation resistant than the parent culture (data not shown). When the process was repeated, again selecting colonies from the "tail" of the previously irradiated cultures (i.e. from the 1.50 kGy dose) and again irradiating (Fig. 3), no significant differences in the regression lines were noted. These results indicate that the observed non-linearity of the *Listeria* irradiation survival curves is probably not the result of differing radiation sensitivities of the cells within the populations, although low mutation rates of the irradiated cells may have precluded selection of true mutant cells.

The effect of menstruum on radiation sensitivity was studied by growing the V7 strain of *L. monocytogenes* in BT medium, centrifuging the cells and resuspending them

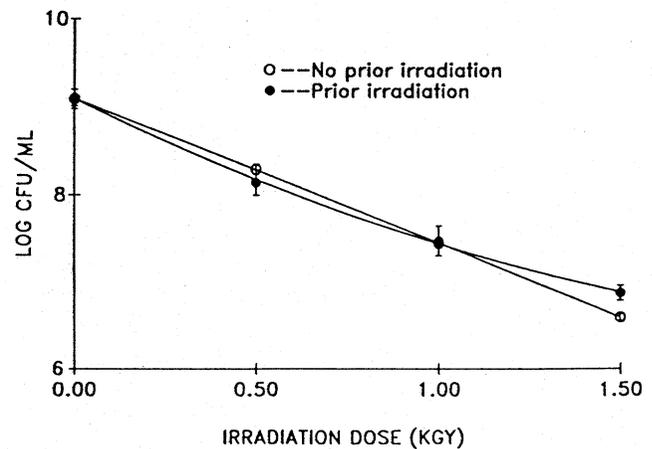


Figure 3. Effect of irradiation on cultures of *L. monocytogenes* selected from colonies on plates of cells exposed to a dose of 1.5 kGy.

in water, buffer (0.026 M KH_2PO_4 and 0.028 M $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$), the original supernatant, or in freshly made BT medium (Fig. 4). At the 1.0 kGy dose the log cfu/ml for the control culture (not centrifuged) was 7.48 (the non-irradiated control was 9.07) while the cells resuspended in the supernatant and in new medium had counts of 5.82 and 5.70, respectively. The centrifuging step resulted in decreased counts of CFU caused perhaps by death of some cells during the process or by clumping. Resuspending in water before irradiation resulted in plate counts below the detection level while resuspending in buffer gave counts that were barely detectable. Radiation sensitivity of bacteria is known to be strongly influenced by the amount of available water in the system (3,9); irradiation of water produces free radicals (15) which exert a damaging "indirect" effect on cellular constituents. Free radicals can be scavenged by organic molecules particularly those containing sulfhydryl groups. This may have been responsible for the observed increase in radiation resistance of *Listeria* when the cells were resuspended in either the supernatant fluid or in fresh media. Increased radiation resistance of clostridia in meat products has been reported (1,9).

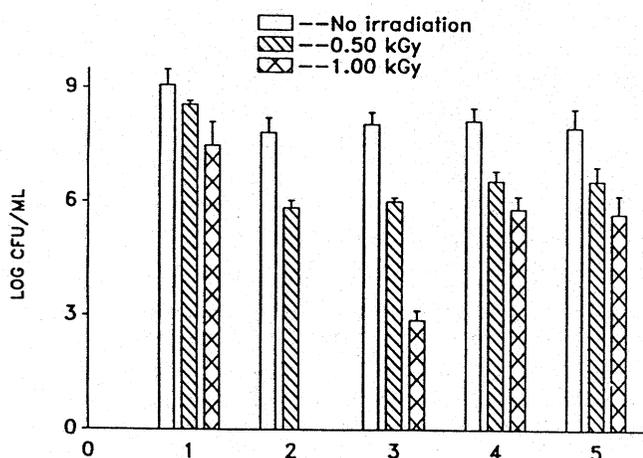


Figure 4. *L. monocytogenes* strain V7 grown 12 h at 35°C in BNT medium, centrifuged at 3000 x g and resuspended to original volume: 1—control culture not centrifuged; 2—cells resuspended in H_2O (counts from 1.00 kGy irradiation were below detection levels); 3—cells resuspended in buffer; 4—cells resuspended in supernatant; 5—cells resuspended in fresh medium.

Our results suggest that gamma radiation would be effective in reducing or eliminating *Listeria* from meat at doses less than 10 kGy; such doses of radiation are considered to be "low". An applied dose of 2.0 kGy would be sufficient to destroy 4 \log_{10} of *Listeria*.

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