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Radiation Resistance of Lactobacilli Isolated from Radurized Meat Relative to Growth and Environment

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Of 113 lactobacilli isolated from radurized (5 kGy) minced meat, 7 *Lactobacillus sake* strains, 1 *L. curvatus* strain, and 1 *L. farciminis* strain were used for radiation resistance studies in a semisynthetic substrate (i.e., modified MRS broth). Five reference *Lactobacillus* spp., one *Staphylococcus aureus* strain, and one *Salmonella typhimurium* strain were used for comparative purposes. All *L. sake* isolates exhibited the phenomenon of being more resistant to gamma-irradiation in the exponential (log) phase than in the stationary phase of their growth cycles by a factor of 28%. Four reference strains also exhibited this phenomenon, with *L. sake* (DSM 20017) showing a 68% increase in resistance in the log phase over the stationary phase. This phenomenon was not common to all bacteria tested and is not common to all strains with high radiation resistance. Four *L. sake* isolates and three reference strains were used in radiation sensitivity testing in a natural food system (i.e., meat). The bacteria were irradiated in minced meat and packaged under four different conditions (air, vacuum, CO_2, and N_2). Organisms exhibited the highest death rate (lowest D_{10} values [doses required to reduce the logarithm of the bacterial population by 1]) under CO_2 packaging conditions, but resistance to irradiation was increased under N_2. The D_{10} values of the isolates were generally greater than those of the reference strains. The D_{10} values were also higher (approximately two times) in meat than in a semisynthetic growth medium.

Ever since its discovery, ionizing radiation has been known to have a lethal effect on microorganisms (10). Microorganisms, however, vary widely in their resistance to ionizing radiation. In general, the gram-negative bacteria, including most of the common food spoilage organisms (e.g., pseudomonads), are more sensitive to irradiation than are the gram-positive organisms (e.g., lactic acid bacteria and micrococci) (7). The lactobacilli therefore tend to dominate the microbial population in radurized meat (12, 15). Their ability to withstand irradiation, coupled with their ability to outcompete other meat-associated microorganisms, causes the lactobacilli to be the most common bacterial group found in vacuum-packaged radurized meat (12). *Lactobacillus sake*, in fact, is the predominant outgrowth organism in vacuum-packaged radurized meat (11; J. W. Hastings and W. H. Holzapfel, Int. J. Food Microbiol., in press).

These facts make the lactobacilli (and specifically *L. sake*) the obvious group to study to gain an understanding of the physiological characteristics of the common bacteria in radurized beef. These characteristics are of obvious technological importance in terms of extending the shelf life of meat. The doses required to reduce the logarithm of the bacterial population by 1) (D_{10}) of different *Lactobacillus* species have been reported previously (W. H. Holzapfel and J. G. Niemand, IAEA/FAO Intl. Symp. on Food Irradiation Processing, 1985, 271/40P, p. 67–68.). In most cases, the D_{10} of an organism is calculated by irradiating an 18- to 24-h culture in a semisynthetic growth medium at ambient temperatures. This, however, may not give a true picture of the resistance of that organism in the medium in which it will be irradiated in commercial practice.

The radiation resistance of a specific organism may vary according to the environment in which it is irradiated (14). Factors that may influence the radiation resistance of bacterial cells include (i) intrinsic factors such as the presence of nutrient-rich substrates (5), (ii) extrinsic factors such as the amount of moisture present (9) or the absence or presence of certain gases in the atmosphere (3, 6, 12) and (iii) implicit factors such as the initial concentration (2) and the age and growth stage of cells (5). Thus far, only one report is known of bacteria that are more resistant to irradiation in the log phase of their growth cycle than in the stationary phase (8). This phenomenon was especially characteristic of very resistant strains.

This study was undertaken to obtain more information on the radiation resistance of lactobacilli isolated from radurized meat, to determine the effects of various environments on the demise of the cells, and to determine the most accurate and applicable method of determining a D_{10} value.

MATERIALS AND METHODS

Isolation and selection of cultures. Minced meat (1 kg) was divided into 20-g portions and vacuum packaged in non-oxygen-permeable pouches. These samples were incubated for 7 days at 5°C. On day 7, all samples were irradiated with a selection dose of 5 kGy. These samples were incubated again at 5°C. Catalase-negative organisms were transferred onto two template agar plates (modified MRS [see below]), of which one was irradiated (2 kGy) and the other was kept as a control. Colonies that survived the 2.0-kGy dose were isolated and used for further testing. The characteristics of these isolates were determined and this led to their identification (Hastings and Holzapfel, in press). All strains were tested for the presence of mesodiaminopimelic acid in the cell wall. This is a distinguishing characteristic for *L. plantarum*. Ten isolates and the nine reference strains (*L. curvatus* DSM 20010, *L. sake* DSM 20017, *L. alimentarius* DSM 20249, *L. plantarum* subsp. *plantarum* DSM 20174, *L. coryniformis* subsp. *coryniformis* DSM 20007, *L. farciminis* DSM 20184, *L. casei* subsp. *pseudoplantarum* DSM 20008,

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Staphylococcus aureus DSM 799, and *Salmonella typhimurium* 712 isolated from chicken) were selected for the semisynthetic medium radiation resistance tests (subsequently referred to as broth radiation resistance tests). DSM refers to Deutsche Sammlung von Mikroorganismen, Gottingen, Federal Republic of Germany, from which these strains were obtained. Four isolates and three reference strains (*L. sake*, *L. curvatus*, and *L. alimentarius*) were selected for the studies in the natural food system (subsequently referred to as meat radiation resistance tests).

Media. The growth medium used was *Lactobacillus* broth (MRS) or agar (Merck and Co., Inc., Rahway, N.J.) that was modified by omitting sodium acetate from the recipe. This medium is referred to as modified MRS. All serial dilutions were done in one-quarter-strength Ringer solution (Merck).

Broth radiation resistance tests. Radiation resistance tests were performed on isolates 1420, 1428, 1432, 4424, 4421, 4420, and 1103 and the nine reference strains mentioned above. Radiation inactivation curves were used to determine D_{10} values for each organism. D_{10} values were calculated both in the log phase and in the stationary phase of the growth cycle of each organism. A radiation resistance curve and the resulting D_{10} values were determined as follows. One milliliter of a 24-h culture was used to inoculate 100 ml of modified MRS broth. The bacteria were then allowed to grow for the desired period, depending upon which stage of the growth cycle was to be tested. The desired period was related to a specific absorbance reading that was determined from growth curves which had previously been calculated by plotting A_{620} over time. The absorbance readings at which the D_{10} evaluations were done were (i) early log phase, 0.300 to 0.400; (ii) log phase, 0.800 to 1.000; (iii) late log phase, 1.500 to 1.700; (iv) stationary phase, always greater than 2.000 after 24 h.

At the desired time, the cultures were irradiated with doses of 1, 2, 3, 4, or 5 kGy. A bacterial count on modified MRS agar was done at each dose level by using one-quarter-strength Ringer solution as a diluent. The logarithm of the bacterial count was plotted against the radiation dose to determine the death rate of the organisms. The curve was plotted by using a standard regression line equation, $y = a + bx$, where y is the log of bacterial survivors, a is the log of total bacteria before irradiation, b is the inactivation rate (in kilograys), and x is the dose (in kilograys). The significance of the regression line is given by the correlation coefficient r. The Student t test was used to check the significance of r. These calculations were done to ensure that the data were not irregular. Results of replicate experiments were used for the calculations.

Meat radiation resistance tests. Only four *L. sake* isolates (1428, 1432, 4424, and 1420) and three of the reference strains (*L. sake*, *L. curvatus*, and *L. alimentarius*) were used in the experiment involving radurized meat. A 100-ml sample of a 24-h culture was mixed thoroughly with 1 kg of sterile minced meat radappertized at a dose of 25 kGy. Portions of 20 g were then placed in non-oxygen-permeable pouches and packaged under the following atmospheres of (i) 90% CO_2:10% N_2, (ii) 100% N_2, (iii) 100% air, and (iv) vacuum. Vacuum packaging was done by using a Kramer and Grebe Autovac Variant IV (Biedenkopf-Wallau, Federal Republic of Germany) (vacuum capacity, 63 m^3/h), measuring a vacuum of 6 mm Hg (799.932 Pa) in a mercury manometer (chamber vacuum, 660 mm Hg [87.993 k Pa]). A broth control was also irradiated with the meat samples, and D_{10} values were calculated as for the broth radiation resistance tests.

TABLE 1. D_{10} values for selected lactobacilli isolated from radurized meat and for several authentic strains in a semisynthetic medium in the log and stationary phases of their growth cycles

Organism	D_{10} (kGy) in:	
	Log phase	Stationary phase
Selected isolates[a]		
L. sake 1420	0.78	0.58
L. sake 4424	0.89	0.58
L. sake 4430	0.59	0.51
L. sake 1428	1.02	0.81
L. sake 1432	0.73	0.61
L. sake 4434	0.76	0.59
L. sake 1103	0.75	0.63
L. sake 4421	1.05	0.79
L. curvatus 1423	0.67	1.47
L. farciminis 3702	1.06	0.72
Authentic strains[b]		
L. plantarum DSM 20174	0.47	0.69
L. farciminis DSM 20184	0.55	0.47
L. alimentarius DSM 20249	0.41	0.59
L. coryniformis DSM 20007	0.76	0.81
L. sake DSM 20017	0.63	0.38
L. casei DSM 20008	0.47	0.45
L. curvatus DSM 20010	0.64	0.53
Staphylococcus aureus 799	0.22	0.29
Salmonella typhimurium 712	0.28	0.56

[a] Average of *L. sake* isolates values: log phase, 0.82 kGy; stationary phase, 0.64 kGy.

[b] Average of authentic lactobacilli values: log phase, 0.53 kGy; stationary phase, 0.56 kGy.

Irradiations. All irradiation processing of meat and bacterial cultures was carried out with a Gamma-beam 650 facility (Atomic Energy of Canada Limited, Ottawa, Ont., Canada) at a dose rate of 19.1 kGy/h for the broth radiation resistance tests. The dose rate was 18.7 kGy/h for the meat radiation resistance tests. The source diameter for both sets of tests was 25 cm.

RESULTS

The broth radiation resistance D_{10} values for all organisms are shown in Table 1. The average D_{10} of the *L. sake* isolates in the stationary phase was 0.64 kGy (range, 0.51 to 0.81) compared with 0.82 kGy (range, 0.59 to 1.05) in the log phase. The average D_{10} value of the seven reference strains in the stationary phase was 0.56 kGy (range, 0.38 to 0.81) and in the log phase was 0.53 kGy (range, 0.41 to 0.76). All of the *L. sake* isolates were more resistant to irradiation in the log phase than in the stationary phase, with an average of 28% increased resistance. Strain 4424 showed the greatest increase in resistance (53%). Of the seven reference strains, four were more resistant in the log phase, with the reference *L. sake* strain (DSM 20017) showing the greatest increase in resistance (68%). The other three reference strains showed the converse result, with *L. plantarum* 47% more resistant in the stationary phase. Both *Staphylococcus aureus* and *Salmonella typhimurium* were more resistant in the stationary phase.

Figure 1 shows the D_{10} values of the four *L. sake* strains and the three reference strains under the various packaging environments (air, vacuum, CO_2, and N_2) and in broth. Table 2 indicates the average D_{10} values of the strains under the conditions outlined above. These results showed (i) that there was a marked increase in resistance to irradiation in the natural environment; (ii) that the strains which were

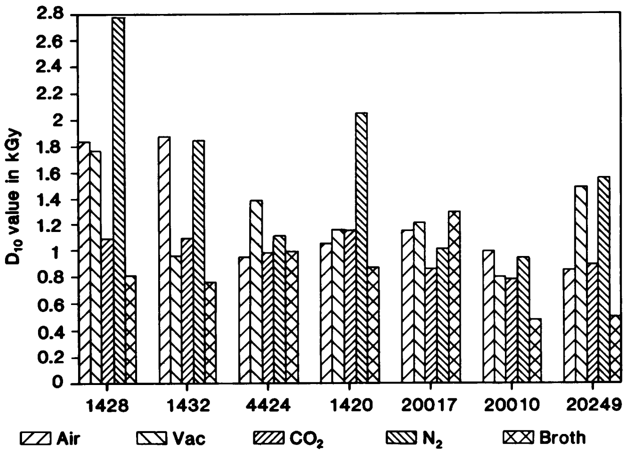

FIG. 1. Histogram of D_{10} values of *L. sake* isolates (strains 1428, 1432, 4424, and 1420), authentic strains of *L. curvatus* DSM 20010, *L. sake* DSM 20017, and *L. alimentarius* DSM 20249 in minced meat (packaged under the given atmospheres) and in broth.

isolated from radurized meat showed greater resistance than did the reference strains; and (iii) that vacuum and CO_2 packaging seemed to decrease resistance, while N_2 had the opposite effect in the majority of cases.

DISCUSSION

The phenomenon of a microorganism's being more resistant to irradiation in the log phase than in the stationary phase of its growth cycle is a little unexpected. It was reported once for *Moraxella* and *Micrococcus* spp. (8). All of the *L. sake* isolates, the *L. farciminis* isolate, and the authentic strains *L. farciminis* DSM 20184, *L. sake* DSM 20017, and *L. curvatus* DSM 20010 exhibited higher radiation-resistance in the log phase than in the stationary phase.

There is not enough information in this study to propose what causes this phenomenon. Certain observations, however, are of interest. First, it is significant that *L. curvatus* 1423, *Staphylococcus aureus*, *Salmonella typhimurium*, and three of the reference strains did not exhibit this characteristic. This proves that it is not common to all bacteria. Second, *L. curvatus* 1423 was more resistant in the stationary phase, whereas the reference strain *L. curvatus* DSM 20010 was more resistant in the log phase. Therefore, the phenomenon may not even be a common characteristic among bacteria of the same species. Third, higher resistance in the log phase was not always related to high radiation resistance, as suggested by previous work (8), because the highly resistant *L. curvatus* 1423 did not exhibit this phenomenon (Table 1).

The D_{10} range for the *L. sake* isolates in the stationary phase (0.51 to 0.81 kGy) was similar to values previously described for these isolates (11). It is interesting that the D_{10} values calculated by a linear regression equation are usually higher than those calculated from the linear section of a drawn curve. For example, for reference strain *L. sake* DSM 20017, D_{10} in the stationary phase was 0.63 kGy from the linear regression equation and 0.43 kGy from the linear section of the drawn curve. The reason for the large decrease is the large shoulder at the beginning of the curve. If a shoulder is present, then the D_{10} value derived from the curve may not give an accurate estimate of the dose required to completely eliminate the organism, although it does give a more accurate estimate of the rate at which the organisms are being killed (after commencement of killing).

It is interesting that the *Salmonella typhimurium* strain used in these experiments showed a higher resistance to irradiation than did the *S. aureus* strain used, in both the log and stationary phases, which is unusual (Holzapfel and Niemand, IAEA/FAO Intl. Symp. on Food Irradiation Processing, 1985). This *Salmonella typhimurium* strain, however, was isolated from chicken and might differ from authentic strains in radiation resistance. This difference is very noticeable when *Salmonella typhimurium* is compared with *L. sake* (Table 1).

Meat radiation resistance tests. The marked increase of resistance to irradiation by the organisms in the natural environment of meat is an expected phenomenon (5; Holzapfel and Niemand, IAEA/FAO Intl. Symp. on Food Irradiation Processing, 1985). The inhibitory effect of vacuum and CO_2 packaging on microorganisms in meat is also well established (1, 4, 13). Nitrogen gas, however, has never been shown to be inhibitory but is often used as a gas filler to overcome exudation and distortion of meat caused by vacuum packaging (4). Most of the previous research indicates that CO_2 and vacuum packaging generally selects for the lactobacilli but decreases their growth rate. Whether or not these conditions affect the initial death rate is the question evoked by these studies. The meat that was packaged under normal atmospheric air was included as a parameter by which to judge the others. The averages (Table 2) of the D_{10} values indicate that in both the isolates and the reference strains, CO_2 packaging (more than all other packaging conditions) seemed to synergize with irradiation to cause an increased death rate (i.e., lower D_{10}). Vacuum-packaging also caused a slight increase in the death rate, but the fact that N_2 packaging increased resistance (in most cases) and lowered the death rate was unexpected. The fact that N_2 buffers some of these bacteria against the lethal effect of irradiation may be of some technological consequence, especially in products in which these organisms are desirable.

These findings therefore suggest the following. (i) When determining the radiation resistance of an organism, reference should be made to its different growth stages. (ii) The radiation resistance value of an organism in a semisynthetic medium may not be a valid criterion to use in setting parameters for a process. Instead, the radiation resistance of

TABLE 2. Average D_{10} values of four *L. sake* strains and three authentic *Lactobacillus* spp. tested in a natural meat environment packaged under air, vacuum, CO_2, or N_2

Strain and atmosphere	Avg D_{10}[a] (kGy)
Isolates[b]	
Air	1.47
Vacuum	1.34
CO_2	1.08
N_2	1.95
Reference strains[c]	
Air	1.00
Vacuum	1.18
CO_2	0.87
N_2	1.15

[a] Average of two experiments.
[b] Isolates were *L. sake* 1428, 1432, 4424, and 1420.
[c] Reference strains were *L. sake* DSM 20017, *L. curvatus* DSM 20010, and *L. alimentarius* DSM 20249.

the organism(s) in the natural substrate should be a valid criterion.

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