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# Radiation Sensitivities of *Listeria monocytogenes* Isolated from Chicken Meat and Their Growth at Refrigeration Temperatures

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***Listeria monocytogenes* were isolated in 5 lots, more than one cell in each 25-g sample of 10 lots of chicken meat, which was obtained from several different areas in Japan. From taxonomic study, the psychrotrophic type of 3 isolates grew well at 4°C on Trypticase soy agar slant, whereas 2 isolates grew poorly. Cells of all isolates were sensitive to $\gamma$-irradiation in phosphate buffer, and the $D_{10}$ values obtained were 0.16 to 0.18 kGy under aerobic irradiation conditions similar to the values of salmonellae. In the chicken meat sample, the $D_{10}$ value obtained was 0.42 kGy the same value as in phosphate buffer under anaerobic irradiation conditions, and the necessary dose for inactivation of *L. monocytogenes* was estimated to be 2 kGy in raw chicken meat below $10^{-4}$ CFU (colony forming unit) per gram. In the storage study of chicken meat which was inoculated with about $3\times10^3$ CFU per gram of *L. monocytogenes*, the psychrotrophic type of the isolates grew quickly at 7 to 10°C storage. However, a dose of 1 kGy was also effective to suppress the growth of *L. monocytogenes* at refrigeration temperatures below 10°C.**

Keywords: *Listeria monocytogenes*, $\gamma$-irradiation, refrigeration temperature, chicken meat, food-borne disease

Chicken meat has been considered microbiologically as one of the most contaminated food products of animal origin. In a previous report (Prachasitthisak *et al.*, 1996), high contamination of coliform bacteria with a few contaminated salmonellae was observed in many samples of raw chicken meat. The crucial problem encountered in the consumption of chicken meat and related meat products is the presence of causative pathogens for food-borne diseases, such as *Salmonella*, *Listeria*, *Staphylococcus*, *Campylobacter*, pathogenic *Escherichia coli* and others (Doyle, 1985). Some of these bacteria can survive after heat treatment during the processing of chicken meat to ready-to-eat products.

The health hazard associated with food-borne *Listeria monocytogenes* was demonstrated by the high mortality during outbreaks of listeriosis. Listeriosis can be more serious or fatal in certain high-risk groups such as unborn babies, infants and those with impaired immune systems (Farber & Peterkin, 1991). There have been several reports on the contamination levels of *L. monocytogenes* in poultry products such as those by Schlech *et al.* (1983) and Pini and Gilbert (1988). In a previous report (Rashid *et al.*, 1992), we also observed some contamination of *L. monocytogenes* in frozen shrimps which were imported from South-Asian countries. It has been reported that *L. monocytogenes* can survive at refrigeration temperature (Palumbo, 1986), and the use of low-temperature storage alone cannot be relied upon to keep meat such as raw chickens safe. Irradiation can decrease the microbial road and may also eliminate specific pathogens as previously reported (Prachasitthisak *et al.*, 1996).

The objectives of this study were to evaluate the contamination level of *L. monocytogenes* in raw chicken meat produced in Japan; to determine the necessary dose of $\gamma$-irradiation to eliminate *L. monocytogenes*; and to observe the irradiation effect on the suppression of growth of *L. monocytogenes* at refrigerated temperatures in chicken meat.

## Materials and Methods

*Sample preparation* Ten different kinds of chicken meat produced in 4 different prefectures were obtained from a wholesale trader for the evaluation of *Listeria* contamination.

*Isolation of L. monocytogenes* Each 25 g sample in duplicate was inoculated into 225 ml of enriched broth [30 g Trypticase soy broth (Difco, Detroit, Michigan), 6 g yeast extract, 15 mg acriflavin HCl, 40 mg nalidixic acid, 50 mg cycloheximide per liter, pH 7.4] and incubated at 30°C for 7 days. One milliliter of the incubated broth was transferred into 9 ml of 0.5% (w/v) KOH solution, mixed well and streaked on selective McBride listeria agar plates [35.5 g phenylethanol agar (Difco), 0.5 g LiCl, 200 mg cycloeximide per liter, pH 7.0] and incubated at 35°C for 48 h (Lovett, 1988). Bluish colonies on the agar plates were selected as probably of the *Listeria* species. Taxonomic characteristics of isolated strains were determined by the method of Cowan & Steel (1974) and Lovett (1988).

*Radiation sensitivities of isolates* For the study of radiation sensitivities in 0.067 M phosphate buffer (pH 7.0) suspensions, representative strains were grown to stationary phase in Tryptic soy broth (containing 0.6% yeast extract)

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under aerobic conditions for 16 h at 30°C. Cell suspensions were prepared with the same buffer to give about $10^9$ cells/ml and irradiated at room temperature in an equilibrium of air or in nitrogen gas at a dose rate of 2.4 kGy/h using a 120 kCi ($4.44\times10^{15}$ Bq) slab-type $^{60}$Co $\gamma$-irradiator. For the study of the inactivation of *L. monocytogenes* in chicken meat, 0.5 ml samples of 16-h cultures in Tryptic soy broth were transferred to each 5 g sample of chicken meat to be about $10^8$ cells/g which had been packed into polyethylene bags and pasteurized by exposure to 10 kGy $\gamma$-rays. These samples were irradiated at 2.4 kGy/h at room temperature. Viable cells were determined by counting visible colonies which developed after diluting the irradiated samples with sterile saline [0.85% (w/v) NaCl] and incubating at 30°C for 1 or 2 days on Tryptic soy agar plates (containing 0.6% yeast extract).

*Radiation effect on the growth* For the study of the radiation effect on the growth of *L. monocytogenes* in chicken meat, 0.5 ml samples of 16-h cultures in Tryptic broth were transferred to 10 g chicken meat to be about $3\times10^3$ CFU (colony forming unit)/g which had been packed into polyethylene bags and pasteurized by exposure to 10 kGy $\gamma$-rays. These samples were irradiated with $\gamma$-rays at 0, 0.5, 1.0 and 1.5 kGy and stored up to 12 to 15 days at 5, 7 and 10°C. Surviving cells were determined by counting visible colonies after homogenization by a Coloworth stomacher Lab-blender-400, the same method as used in the previous report (Prachasitthisak *et al.*, 1996).

## Results

In this study, *L. monocytogenes* were isolated in 5 lots, one or two cells in each 25-g sample from 10 lots of chicken meat. However, detection of *L. monocytogenes* was made in only one sample in each 5 lots of duplicate samples. As shown in Table 1, three isolates belonged to the psychrotrophic type which could grow well on the Tryptic soy agar slant at 4°C. However, two isolates could not grow well on the agar slant at 4°C.

Figure 1 shows the radiation survival curves of *L. monocytogenes* G2-1 exposed by $\gamma$-irradiation in phosphate buffer under aerobic and anaerobic conditions. All of the isolates had similar sigmoidal type of survival curves which exhibited a shoulder, and the $D_{10}$ values obtained were 0.16 to 0.18 kGy under aerobic conditions, values similar to those of salmonellae (Prachasitthisak *et al.*, 1996). However, the

**Table 2.** Radiation sensitivities of typical isolates of *Listeria monocytogenes* under air-equilibrium and nitrogen-gas atmosphere in 0.067 M phosphate buffer.

| Isolate | $D_{10}$ value under air-equilibrium | $D_{10}$ value under nitrogen-gas atmosphere |
|---|---|---|
| I1-1 | 0.16 kGy | 0.42 kGy |
| I2-2 | 0.18 kGy | 0.42 kGy |
| I4-1 | 0.17 kGy | 0.42 kGy |
| G2-1 | 0.16 kGy | 0.42 kGy |

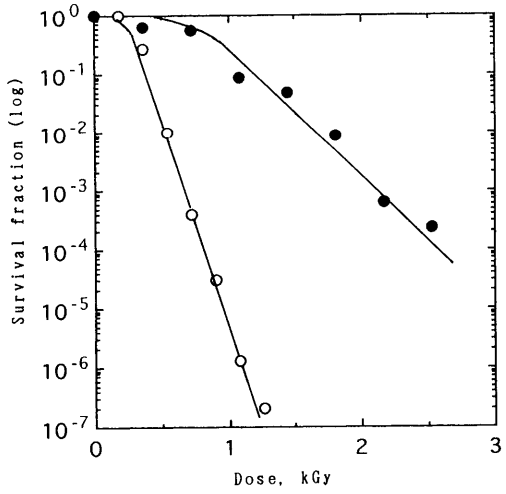


**Fig. 1.** Radiation sensitivities of *L. monocytogenes* G2-1 under air-equilibrium (○) and nitrogen-gas atmosphere (●) in 0.067 M phosphate buffer.

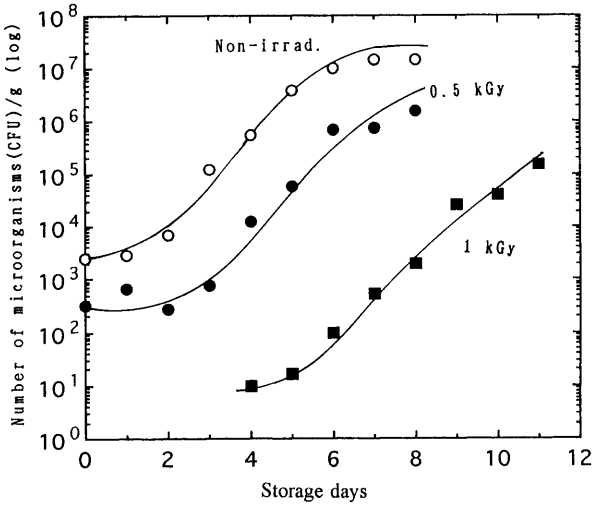


**Fig. 2.** Growth of *L. monocytogenes* G2-1 in chicken meat at 10°C after irradiation at 0, 0.5 and 1.0 kGy.

**Table 1.** Characteristics of isolates of *Listeria monocytogenes* from chicken meat.

| Isolate | Catalase | Beta hemolysis | Nitrate reduction | Urea hydrolysis | Glucose fermentation | Growth at 4°C |
|---|---|---|---|---|---|---|
| I1-1 | +/− | ++ | − | − | +++ | ++ |
| I2-2 | + (weak) | +++ | − | − | +++ | +/− |
| I4-1 | +/− | ++ | − | − | +++ | ++ |
| G2-1 | +/− | +++ | − | − | +++ | ++ |
| M2-1 | + (weak) | ++ | − | − | +++ | +/− |

All isolates are gram-positive, short rods (0.5×0.5−0.7 $\mu$m), motile, and oxidase negative.

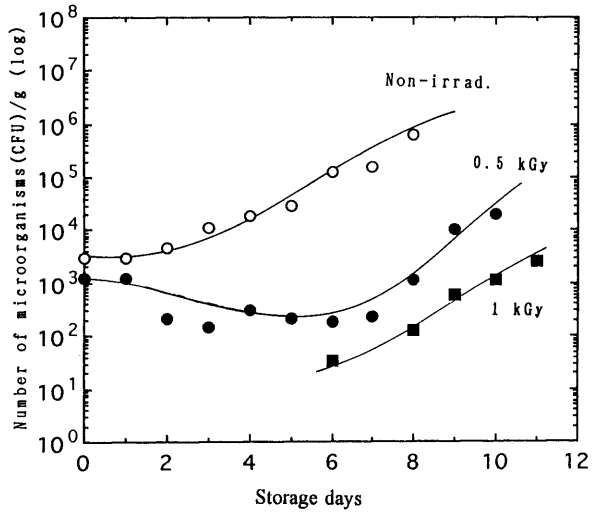


**Fig. 3.** Growth of *L. monocytogenes* G2-1 in chicken meat at 7°C after irradiation at 0, 0.5 and 1.0 kGy.

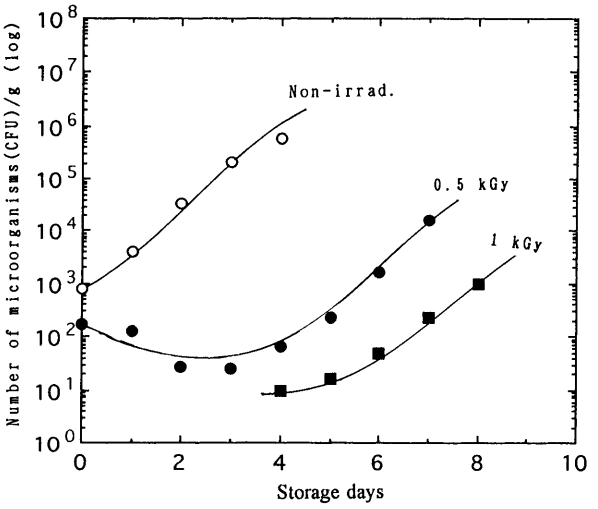


**Fig. 4.** Growth of *L. monocytogenes* I2-2 in chicken meat at 10°C after irradiation at 0, 0.5 and 1.0 kGy.

same $D_{10}$ value of 0.42 kGy was obtained at all of the isolates under anaerobic irradiation conditions as shown in Table 2. The difference in the radiation sensitivities between aerobic and anaerobic conditions should be explained by the oxygen effect of radiation which had been observed in many types of bacteria in a previous report (Ito *et al.*, 1972). The $D_{10}$ value obtained in chicken meat was also 0.42 kGy on the G2-1 and I2-2 strains, the same value as under anaerobic conditions in phosphate buffer with the same survival curve. From these results, 2 kGy should be adequate as the necessary dose to inactivate *L. monocytogenes* in chicken meat below $10^{-4}$ CFU/g.

The growth of *L. monocytogenes* G2-1 which belong to the psychrotrophic type in chicken meat occurred significantly at 10°C storage as shown in Fig. 2. The initial load of G2-1 strain with about $3\times10^3$ CFU/g increased quickly during 2 to 6 days storage till to about $10^7$ CFU/g at 10°C. The growth of the G2-1 strain at 7°C storage was also significantly observed as shown in Fig. 3. However, its growth at 5°C occurred slowly even after 10 days storage which increased up to $1\times10^5$ CFU/g. In the case of the I2-2 strain which does not belong to the psychrotrophic type, its growth at 10°C occurred somewhat slower than the G2-1 strain as shown in Fig. 4, and the growth at 7°C occurred slowly even after 10 days storage, similar to the G2-1 strain at 5°C storage.

The suppressive growth effect by irradiation on the G2-1 strain at 10°C was clearly observed at 1 kGy for 4 to 6 days storage. However, no growth was observed even when stored up to 15 days at 1.5 kGy irradiation on chicken meat. At 7°C storage, growth delay by irradiation on the G2-1 strain was observed even at 0.5 kGy as shown in Fig. 3. A similar effect of growth delay by irradiation was also observed on the I2-2 strain at 10°C storage, which suppressed the growth even at 0.5 kGy as shown in Fig. 4. The effect of growth delay on the I2-2 strain after irradiation at 7°C storage and on the G2-1 strain after irradiation at 5°C storage could not be clearly observed during 15 days storage because of the slow growth in chicken meat even for non-irradiated samples. The suppressive effect on the growth of *L. monocytogenes* in irradiated samples at refrigerated temperatures should be attributed to the suppression of the repair of radiation-injured cells at lower temperatures (Ito *et al.*, 1972).

## Discussion

Recently, food-borne diseases by salmonellae or pathogenic *E. coli* O157:H7 are significantly increasing in Japan. These bacteria have been replaced from *Vibrio parahaemolyticus* which had been one of a most common food-poisoning bacteria in Japan more than 5 to 10 years ago. These increases of food-borne diseases should be attributed to the change in food customs of consumers and to the invading new type of pathogens from abroad. In the future, food poisoning by *Listeria* should cause some problems in Japan just as abroad (Doyle, 1985).

From this study, the contamination level of *L. monocytogenes* in chicken meat in Japan was observed to be 50%, which is lower than that of the USA or UK (Schlech *et al.*, 1983; Pini & Gilbert, 1988), and the amount of contamination of *L. monocytogenes* should be below one cell per g in chicken meat from the results of the low numbers of contamination in 5 lots in each 25-g sample from 10 lots of chicken meat. In this study, psychrotrophic type of isolates could grow significantly at 7 to 10°C storage in raw chicken meat just as coliform bacteria (Prachasitthisak *et al.*, 1996). The results of radiation sensitivities of many isolates of *L. monocytogenes* revealed that the $D_{10}$ values and survival curves are not significantly different from the isolates, and a dose of 2 kGy should be sufficient to completely inactivate low numbers of this bacterium and other hazardous pathogens such as pathogenic *E. coli*, salmonellae in chicken meat similar to the results of Thayer and Boyd (1993), Kamat and Nair (1995) and Murano (1995). However, a dose of 1 kGy is also effective in suppressing the growth of *L. monocytogenes* at refrigeration temperatures similar to coliform bacteria in chicken meat which was previously reported (Parachasitthisak *et al.*, 1996). From these results, a low-dose irradiation at 1 kGy followed

by refrigeration should be effective for the reduction of the initial microbial load, the extension of shelf life and the improvement of safety on food poisoning bacteria without affecting any sensory quality of raw chicken meat by radiation.

**References**


Cowan, S.T. and Steel, K.J. (1974). "Identification of Medical Bacteria," 2nd ed., Cambridge University Press, Cambridge.

Doyle, M.P. (1985). Food borne pathogen of recent concern. *Ann. Rev. Nutr.*, **5**, 25-34.

Farber, J.M. and Peterkin, P.I. (1991). *Listeria monocytogenes*: A food borne pathogen. *Microbiol. Rev.*, **55**, 476-571.

Ito, H., Iizuka, H., Okazawa, Y. and Watanabe, H. (1972). Radiosensitivity of a radio-resistant strain of Pseudomonas radiora and its recovery from radiation damage. *Nogei Kagaku Kaishi*, **46**, 127-135 (in Japanese).

Kamat, A.S. and Nair, M.P. (1995). Gamma irradiation as a means to eliminate *Listeria monocytogenes* from frozen chicken meat. *J. Sci. Food Agric.*, **69**, 415-422.

Lovett, J.R. (1988). Isolation and enumeration of *Listeria monocytogenes*. *Food Technol.*, **42**, April, 172-175.

Murano, E.A. (1995). Irradiation of fresh meat. *Food Technol.*, **52**, December, 52-54.

Palumbo, S.A. (1986). Is refrigeration enough to restrain food borne pathogen, *J. Food Prot.*, **49**, 1003-1009.

Pini, P.N. and Gilbert, R.J. (1988). A comparison of two procedures for the isolation of *Listeria monocytogenes* from raw chickens and soft cheese. *Int. J. Food Microbiol.*, **7**, 331-337.

Prachasitthisak, Y., Banati, D. and Ito, H. (1996). Shelf life extension of chicken meat by $\gamma$-irradiation and microflora changes. *Food Sci. Technol. Int.*, **2**, 242-245.

Rashid, H.O., Ito, H. and Ishigaki, I. (1992). Distribution of pathogenic vibrios and other bacteria in imported frozen shrimps and their decontamination by gamma-irradiation. *World J. Microbiol. Biotech.*, **8**, 494-499.

Schlech, W.F., Lavigne, P.M., Bortolussi, R.A., Allen, A.C., Haldane, E.V., Wort, A.J., Hightower, A.W., Johnson, S.E., King, S.H., Nicholls, E.S. and Broome, C.V. (1983). Epidemic listeriosis evidence for transmission by food. *N. Engl. J. Med.*, **308**, 202-208.

Thayer, D.W. and Boyd, G. (1993). Elimination of *Escherichia coli* O157; H7 in meat by gamma irradiation. *Appl. Environ. Microbiol.*, **59**, 1030-1034.