Screening of Radio-resistant Lactic Acid Bacteria

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Abstract

This study screened for radio-resistant strains lactic acid bacteria (LAB) by evaluating their capability to survive exposure to ionizing radiation. Ten strains of LAB - *Lactobacillus bulgaricus*, *Lactobacillus paracasei*, *Lactobacillus casei*, *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus delbruekii*, *Lactococcus lactis*, *Streptococcus thermophilus*, *Bifidobacterium breve*, and *Pediococcus pentosaceus* - were selected and subcultured twice. The LAB was then further cultured for 3 d at 37°C to reach 7-10 Log colony-forming units (CFU)/mL prior to irradiation and immediately exposed to gamma rays or electron beams with absorbed doses of 0, 1, 2, 3, 4, 5, 6, 8, and 10 kGy. Gamma irradiation gradually decreased the number of the tested viable LAB, and the effect was irradiation dose dependent. A similar effect was found in electron beam-irradiated LAB. Radiation sensitivity of LAB was calculated as D₁₀ values, which ranged from 0.26 kGy to 0.9 kGy and 0.5 kGy to 1.44 kGy with exposure to gamma and electron beam irradiation, respectively, in all tested LAB. *L. acidophilus* was the most resistant to gamma and electron beam irradiation, with D₁₀ values of 0.9 kGy and 1.44 kGy, respectively. These results suggest that *L. acidophilus* might be suitable for the preparation of probiotics as direct-fed microbes for astronauts in extreme space environments.

Key words: ionizing irradiation, lactic acid bacteria (LAB), *Lactobacillus acidophilus*, radio-resistant

Introduction

Lactic acid bacteria (LAB) are microorganisms that have been used extensively in the preparation of fermented food products such as cheese, bread, yogurt, butter, and vinegar. These bacteria have also been used to extend the shelf life of foods by eliminating or reducing the growth of harmful pathogens during storage.

The most commonly used LAB are species of *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, and *Enterococcus* (Parvez *et al.*, 2006). These microorganisms are naturally present in the gastrointestinal microecology of healthy individuals and have been shown to improve intestinal health, reduce symptoms of lactose intolerance, and lower serum cholesterol, which decreases the risk of certain cancers and enhances immunity (Isolauri *et al.*, 2004).

Owing to these beneficial effects of LAB, their use in the food industry is increasing. Fermented milk products were introduced commercially in Korea in 1971, and the sale of products using LAB generated an estimated half a

billion dollars in 2010. The market share of these products is expected to grow at an annual rate of 10% (Youn and Jeong, 2010). This increase is primarily attributable to consumer demand for natural materials as sources of products for prolonged physical well-being and their recognition that health foods have therapeutic benefits including prophylaxis for some intestinal infections (Arendt *et al.*, 2011; Clark *et al.*, 2012; Kumar *et al.*, 2012; Mercenier *et al.*, 2002). The Korea Food and Drug Administration (2012) has also designated probiotics using LAB as health supplement foods. Therefore, the development of new probiotic products through the screening of bio-functional activities from useful LAB is expected to increase with their increasing market values.

LAB can be helpful in maintaining astronaut health. Safety from infection in space environments is one of the main issues in space crew medical support (Ilyin, 2005). Although the susceptibility of astronauts to infection with pathogenic bacteria during space flights has not been confirmed, reports have demonstrated sharp decreases in LAB such as intestinal lactobacilli and bifidobacteria (Ilyin, 2005) and a need for greater concentrations of chemotherapeutic agents (i.e., antibiotics) (Leys *et al.*, 2004), both of which can result in the bodily deterioration of astronauts during missions. LAB is a biotherapeutic agent

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that suppresses the increase in virulence during microfloral proliferation. Providing these probiotics to astronauts during space flight may improve their capabilities under extremely stressful space conditions such as exposure to microgravity or low doses of cosmic rays. However, exposure to cosmic rays might inactivate or mutate LAB (Horneck *et al.*, 1989) and thus affect their beneficial effects in humans. Therefore, the identification of radiation-resistant LAB strains is required before probiotics can be prepared as direct-fed microbes for astronauts.

In this study, LAB used for the production of fermented milk products were tested for their capability to survive exposure to ionizing radiation, and were screened for the selection of radiation-resistant strains suitable for the preparation of probiotics as direct-fed microbes for astronauts in extreme space environments.

Materials and Methods

Strains and culture medium

Ten LAB stains - *Lactobacillus delbrueckii* subsp. *bulgaricus* (KCTC3635), *Lactobacillus paracasei* (KCTC 13169), *Lactobacillus casei* (KCTC3109), *Lactobacillus acidophilus* (KCTC3140), *Lactobacillus plantarum* (KCTC 3103), *Lactobacillus delbrueckii* (KCTC13730), *Lactococcus lactis* (KCTC2013), *Streptococcus salivarius* subsp. *thermophilus* (KCTC3658), *Bifidobacterium breve* (KCTC 3419), *Pediococcus pentosaceus* (KCTC3116) - were obtained from the Korean Collection for Type Cultures (KCTC) at the Korea Research Institute of Bioscience and Biotechnology (Korea).

Brain Heart Infusion (BHI) broth (Difco, USA) was used for the culture of *S. thermophilus* and *L. lactis*, and de Man, Rogosa, and Sharpe (MRS) broth (Difco) was used for the culture of the other 8 strains. The LAB strains were frozen and stored at -70°C in 10% glycerol containing BHI or MRS broth until use.

Preparation of LAB samples

Ten LAB strains were subcultured twice in MRS or BHI broth. Then, 1% subculture LAB suspensions were inoculated and cultured for 3 d at 37°C to reach 7-10 Log colony-forming units (CFU)/mL. The LAB culture suspensions obtained were divided into 1-mL aliquots in Eppendorf tubes. Each of the LAB aliquot samples was exposed to gamma or electron beam irradiation.

Gamma and electron beam irradiation

LAB samples were irradiated with gamma or electron

beams at doses of 0, 1, 2, 3, 4, 5, 6, 8, and 10 kGy. For gamma irradiation, a cobalt-60 irradiator (IR-79; Nordion International Ltd., Canada) with a source strength of 11.1 PBq was used. For electron beam irradiation, an ELV4-electron accelerator (energy, 10 MeV; beam power, 570 kW) was used with a beam current of 1 mA. Dosimetry was performed using an alanine dosimeter (5 mm; Bruker Instruments, Germany) for gamma irradiation and a ceric-cerous dosimeter (Bruker Instruments, Germany) for electron beam irradiation, respectively. The error of the total adsorbed doses in the LAB samples was within 5%. The irradiated LAB samples were immediately subjected to viability tests.

Viability test of irradiated LAB

After irradiation, LAB samples were serially diluted in sterilized saline, plated on solid MRS medium, and incubated for 3 d at 37°C. *Streptococcus thermophilus* and *L. lactis* were incubated on solid BHI medium. LAB viability was expressed as mean Log CFU (suspension mL) ± standard deviation of triplicate measurements. D₁₀ values, defined as radiation dose (kGy) required to reduce the number of CFU by 1 Log₁₀, were determined by calculating the negative reciprocal of the slope of the linear regression curve (Aziz *et al.*, 1997; Bari *et al.*, 2003; Rajkowski *et al.*, 2003).

Statistical analysis

Statistical analysis of the data was carried out with 1-way analysis of variance using SPSS 19.0 software (SPSS Inc, USA), and significantly different means were compared using the multiple range test of Duncan. A p value of <0.05 was considered statistically significant.

Results and Discussion

Irradiation effect on viability of LAB

The effects of gamma irradiation on the viability of the 10 LAB strains studied is provided in Table 1. The viability of non-irradiated LAB cultivated for 3 d ranged from a mean 7.3 Log CFU/mL to a mean 10.9 Log CFU/mL. Gamma irradiation significantly decreased the viability of LAB ($p < 0.05$), and LAB viability decreased with increases in gamma radiation dose. The viability of all LAB strains tested was abrogated at 5.0 kGy with the exception of *L. acidophilus*, which was abrogated at 10.0 kGy. This result agrees with a previous report that *L. acidophilus* is not easily sterilized by exposure to ionizing radiation (Morichi, 1997). Therefore, *L. acidophilus* was con-

Table 1. Radiation sensitivity of lactic acid bacteria against gamma ray

Lactic acid bacteria	Irradiating dose (kGy)	Viable cell No. (Log CFU/mL)
<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	0	7.47±0.02 ^a
	1	5.81±0.04 ^b
	2	4.66±0.19 ^c
	3	2.99±0.09 ^d
	4	1.27±0.03 ^e
	5	ND
<i>Lactobacillus paracasei</i>	0	10.97±0.10 ^a
	1	6.74±0.11 ^b
	2	4.84±0.04 ^c
	3	2.75±0.21 ^d
	4	1.15±0.21 ^e
	5	ND
<i>Lactobacillus casei</i>	0	8.81±0.04 ^a
	1	8.23±0.06 ^b
	2	6.51±0.03 ^c
	3	3.82±0.18 ^d
	4	1.00±0.01 ^e
	5	ND
<i>Lactobacillus acidophilus</i>	0	9.67±0.04 ^a
	1	8.92±0.06 ^b
	2	8.37±0.02 ^c
	3	6.94±0.04 ^d
	4	5.86±0.15 ^e
	6	2.75±0.05 ^f
	8	0.97±0.03 ^g
	10	ND
<i>Lactobacillus plantarum</i>	0	9.69±0.05 ^a
	1	7.17±0.04 ^b
	2	4.29±0.15 ^c
	3	3.57±0.04 ^d
	4	0.15±0.21 ^e
	5	ND
<i>Lactobacillus delbrueckii</i>	0	7.38±0.12 ^a
	1	6.58±0.11 ^b
	2	6.10±0.12 ^c
	3	2.15±0.21 ^d
	4	1.43±0.49 ^e
	5	ND
<i>Lactococcus lactis</i>	0	8.95±0.27 ^a
	1	5.85±0.06 ^b
	2	4.50±0.13 ^c
	3	3.97±0.35 ^d
	4	ND
<i>Streptococcus salivarius</i> subsp. <i>theromophilus</i>	0	9.14±0.07 ^a
	1	7.28±0.14 ^b
	2	6.22±0.05 ^c
	3	3.65±0.07 ^d
	4	0.38±0.12 ^e
	5	ND
<i>Bifidobacterium breve</i>	0	10.65±0.05 ^a
	1	4.93±0.02 ^b
	2	2.87±0.15 ^c
	3	ND

Table 1. Continued

Lactic acid bacteria	Irradiating dose (kGy)	Viable cell No. (Log CFU/mL)
<i>Pedicoccus pentosaceus</i>	0	9.34±0.01 ^a
	1	8.66±0.08 ^b
	2	7.45±0.06 ^c
	3	5.01±0.16 ^d
	4	2.37±0.02 ^e
	5	ND

firmed to be the most resistant to gamma irradiation among the 10 LAB strains tested.

In a parallel experiment, the effects of electron beam irradiation on LAB viability was measured (Table 2). The viability of the non-irradiated LAB cultivated for 3 d ranged from a mean 7.2 Log CFU/mL to a mean 10.2 Log CFU/mL. Similar to gamma irradiation, electron beam irradiation significantly decreased ($p<0.05$) the viability of these LAB and the rate of decreased viability was positively correlated with the increase in electron beam radiation dose. However, unlike gamma irradiation, electron beam irradiation abrogated the viability of LAB at 6.0 kGy with the exception of *L. acidophilus* and *P. pentosaceus*. The latter was sterilized at 8.0 kGy, but the former was not eliminated at even 10.0 kGy. This result demonstrates that *L. acidophilus* is highly resistant to gamma irradiation as well as electron beam irradiation.

Radiation sensitivity of LAB

D_{10} values of the 10 studied LAB strains exposed to gamma irradiation are provided in Table 3. The D_{10} values of these bacteria ranged from 0.26 kGy and 0.90 kGy in a rank order of *L. acidophilus* (0.90 kGy) > *L. bulgaricus* (0.66 kGy) > *L. lactis* (0.61 kGy) = *L. delbrueckii* (0.61 kGy) > *P. pentosaceus* (0.57 kGy) > *L. casei* (0.51 kGy) > *S. thermophilus* (0.47 kGy) > *L. plantarum* (0.44 kGy) > *B. breve* (0.26 kGy).

D_{10} values of LAB exposed to electron beam irradiation were also calculated (Table 4) and ranged from 0.50 to 1.44 kGy. The rank order of D_{10} values of electron beam-irradiated LAB was *L. acidophilus* (1.44 kGy) > *B. breve* (0.82 kGy) > *P. pentosaceus* (0.80 kGy) > *L. casei* (0.68 kGy) > *L. paracasei* (0.64 kGy) > *S. thermophilus* (0.59 kGy) = *L. delbrueckii* (0.59 kGy) > *L. lactis* (0.57 kGy) > *L. plantarum* (0.50 kGy) = *L. bulgaricus* (0.50 kGy).

Previous reports have indicated that the type of LAB strain and the radiation source affect the radiation sensitivity of LAB. For example, Hastings *et al.* (1986) have shown that D_{10} values of *L. plantarum* and *L. casei* isolated from gamma-irradiated minced meats are 0.46 kGy,

Table 2. Radiation sensitivity of lactic acid bacteria against electron beam

Lactic acid bacteria	Irradiating dose (kGy)	Viable cell No. (Log CFU/mL)
<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	0	7.66±0.10 ^a
	1	5.64±0.23 ^b
	2	3.41±0.10 ^c
	3	1.68±0.25 ^d
	4	ND
<i>Lactobacillus paracasei</i>	0	10.21±0.29 ^a
	1	10.10±0.04 ^a
	2	7.13±0.04 ^b
	3	5.94±0.11 ^c
	4	4.37±0.02 ^d
	5	2.58±0.01 ^e
	6	1.49±0.20 ^f
<i>Lactobacillus casei</i>	8	ND
	0	9.17±0.05 ^a
	1	8.83±0.14 ^b
	2	6.73±0.03 ^c
	3	5.39±0.12 ^d
	4	3.59±0.06 ^e
	5	2.27±0.17 ^f
<i>Lactobacillus acidophilus</i>	6	ND
	0	8.70±0.06 ^a
	1	8.65±0.07 ^a
	2	6.82±0.07 ^b
	3	6.03±0.02 ^c
	4	4.86±0.07 ^d
	5	4.83±0.07 ^d
	6	4.58±0.13 ^e
<i>Lactobacillus plantarum</i>	8	2.89±0.04 ^f
	10	1.95±0.16 ^g
	0	7.66±0.10 ^a
	1	5.64±0.23 ^b
	2	3.41±0.10 ^c
<i>Lactobacillus delbrueckii</i>	3	1.68±0.25 ^d
	4	ND
	0	9.12±0.01 ^a
	1	8.79±0.01 ^b
	2	5.87±0.33 ^c
	3	4.78±0.10 ^d
<i>Lactococcus lactis</i>	4	2.79±0.01 ^e
	5	1.00±0.00 ^f
	6	ND
	0	8.82±0.07 ^a
	1	5.17±0.06 ^b
	2	3.97±0.08 ^c
<i>Streptococcus salivarius</i> subsp. <i>theromophilus</i>	3	2.40±0.05 ^d
	4	1.50±0.35 ^e
	5	ND
	0	7.25±0.08 ^a
	1	5.77±0.05 ^b
	2	3.20±0.07 ^c
	3	2.44±0.09 ^d
	4	ND

Table 2. Continued

Lactic acid bacteria	Irradiating dose (kGy)	Viable cell No. (Log CFU/mL)
<i>Streptococcus salivarius</i> subsp. <i>theromophilus</i>	0	7.25±0.08 ^a
	1	5.77±0.05 ^b
	2	3.20±0.07 ^c
	3	2.44±0.09 ^d
	4	ND
<i>Bifidobacterium breve</i>	0	8.43±0.01 ^a
	1	8.15±0.03 ^a
	2	6.04±0.03 ^b
	3	4.84±0.05 ^c
	4	3.79±0.09 ^d
	5	2.70±0.65 ^e
<i>Pedococcus pentosaceus</i>	6	ND
	0	9.43±0.22 ^a
	1	8.93±0.01 ^b
	2	6.96±0.01 ^c
	3	5.92±0.05 ^d
	4	4.99±0.05 ^e
	5	3.47±0.01 ^f
	6	2.06±0.11 ^g
	8	ND

and Byun (2009) have found that D_{10} values of *Lactobacillus* spp. varied from 0.23 kGy to 0.38 kGy. In this study, D_{10} values in the 10 LAB strains varied from 0.26 kGy to 0.90 kGy with exposure to gamma irradiation and from 0.50 kGy to 1.44 kGy with exposure to electron beam irradiation. In particular, the D_{10} values of *L. acidophilus*, *P. pentosaceus*, and *B. breve* greatly increased from 0.90 kGy, 0.57 kGy, and 0.26 kGy, respectively, with exposure to gamma irradiation to 1.44 kGy, 0.80 kGy, and 0.82 kGy, respectively, against electron beam irradiation (Tables 3 and 4). This difference reflects that even LAB with identical sensitivity to radiation can display differences related to the temperature at which irradiation occurs, culture medium composition, growth phase, oxygen content during cultivation, and other conditions (Molins, 2001).

Our results show that among 10 LAB, *L. acidophilus* was the most radio-resistant strain. *Lactobacillus acidophilus* is one of many common probiotics used for the preparation of fermented milk products, and it has diverse biological activities. The LAB examined in our study can suppress the growth of a variety of harmful pathogens (Aween *et al.*, 2012; Millette *et al.*, 2007) and alleviate irritable colon syndrome (Sinn *et al.*, 2008). These bacteria also induce immune-modulating activities such as T-cell immune response (Wen *et al.*, 2012), chemokine production (Jiang *et al.*, 2012), and anti-cancer immune responses (Chen *et al.*, 2012; Maroof *et al.*, 2012). Moreover, *L. acidophilus* can reduce cholesterol absorption in

Table 3. D₁₀ value of lactic acid bacteria by gamma-irradiation

Lactic acid bacteria	D ₁₀ (kGy)	R ²
<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	0.66	0.99
<i>Lactobacillus paracasei</i>	0.42	0.96
<i>Lactobacillus casei</i>	0.51	0.51
<i>Lactobacillus acidophilus</i>	0.90	0.96
<i>Lactobacillus plantarum</i>	0.44	0.97
<i>Lactobacillus delbrueckii</i>	0.61	0.61
<i>Lactococcus lactis</i>	0.61	0.89
<i>Streptococcus salivarius</i> subsp. <i>theromophilus</i>	0.47	0.96
<i>Bifidobacterium breve</i>	0.26	0.93
<i>Pedococcus pentosaceus</i>	0.57	0.94

Table 4. D₁₀ value of lactic acid bacteria by electron beam-irradiation

Lactic acid bacteria	D ₁₀ (kGy)	R ²
<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	0.50	0.90
<i>Lactobacillus paracasei</i>	0.64	0.98
<i>Lactobacillus casei</i>	0.68	0.98
<i>Lactobacillus acidophilus</i>	1.44	0.95
<i>Lactobacillus plantarum</i>	0.50	0.99
<i>Lactobacillus delbrueckii</i>	0.59	0.98
<i>Lactococcus lactis</i>	0.57	0.93
<i>Streptococcus salivarius</i> subsp. <i>theromophilus</i>	0.59	0.96
<i>Bifidobacterium breve</i>	0.82	0.98
<i>Pedococcus pentosaceus</i>	0.80	0.98

cells (Huang and Zheng, 2010), ameliorate *H. pylori*-induced gastric inflammation (Yang *et al.*, 2012), and induce antidiabetic effects in combination with *L. casei* (Yadav *et al.*, 2007).

LAB are safe microorganisms with a long history of use in the preparation of popular fermented milk products. These bacteria also have health-promoting advantages via various biological mechanisms. The number of fermented milk products containing these bio-functional properties is increasing.

In this study, 10 LAB used in the production of fermented milk products were tested for their capability to survive exposure to ionizing radiation. Among those LAB strains, *L. acidophilus* was the most radio-resistant to gamma and electron beam irradiation. These results suggest that *L. acidophilus* might be a suitable candidate for the preparation of probiotics as direct-fed microbes for astronauts in extreme space environments.

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