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Antioxidative Effects of Intracellular Extracts of Yogurt Bacteria on Lipid Peroxidation and Intestine 407 Cells

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ABSTRACT

The antioxidative activity of intracellular extracts of yogurt bacteria was measured based on the inhibition of lipid peroxidation of two model systems, namely a free fatty acid (linoleic acid) system and a biological lipid (plasma lipid) system. The inhibitory rate on linoleic acid peroxidation was 61 and 57% for *Streptococcus salivarius* ssp. *thermophilus* ATCC 19258 and *Lactobacillus delbrueckii* ssp. *bulgaricus* ATCC 11842, respectively. The inhibitory rate on plasma lipid peroxidation was 57 and 41% for *S. salivarius* ssp. *thermophilus* ATCC 19258 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842, respectively. Results from both systems demonstrated that these two yogurt strains were potentially highly antioxidative. Further experiments were done on Intestine 407 cells to determine the antioxidative effects of yogurt bacteria on reducing oxidative damage of oxidant H₂O₂. Intracellular extracts of yogurt bacteria reduced the genotoxicity of H₂O₂. Although *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 had only minor effects on reducing DNA damage, *S. salivarius* ssp. *thermophilus* ATCC 19258 showed excellent potential for protecting the Intestine 407 cells from the genotoxicity of oxidant H₂O₂. Both yogurt bacteria demonstrated high cytotoxicity inhibitory ability for oxidant H₂O₂. The cytotoxicity inhibition rate was 71 and 48% for *S. salivarius* ssp. *thermophilus* ATCC 19258 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842, respectively.

Key words: yogurt bacteria, *Streptococcus salivarius* ssp. *thermophilus*, *Lactobacillus delbrueckii* ssp. *bulgaricus*, antioxidative effects.

INTRODUCTION

Fermented dairy foods containing lactic acid bacteria have been consumed for centuries in Western countries. Today, these products are becoming popular in other parts of the world due to their benefits for human health. Yogurt, which is fermented with *S. salivarius* ssp. *thermophilus* and *L. delbrueckii* ssp. *bulgaricus*, is one of the most popular fermented foods. It not only is nutritious but also has been reported to be probiotic. The reported nutritional and health benefits of cultured yogurt include: hypoallergenic effects toward milk protein, enhancement of bioavailability of calcium and other nutrients, improvement of lactose intolerance, presence/colonization of active culture in the GI tract, control of intestinal infections, stimulation of the immunological system, anticarcinogenic effects, growth stimulation, reduction of serum cholesterol, and longevity⁽¹⁾. Besides these probiotic effects, the antioxidative ability of lactic acid bacteria, including yogurt bacteria, has been reported lately⁽²⁻⁶⁾. Among these studies, Kaizu *et al.*⁽³⁾ were the first to demonstrate that intracellular extract of *Lactobacillus* sp. SBT 2028 was antioxidative.

Although oxidation is essential to living organisms for the production of energy to fuel biological processes, oxidative stress can damage biological molecules. It is

well-established that a wide variety of oxygen-centered free radicals and other reactive oxygen species are continuously produced in the human body and in food systems⁽⁷⁾. Oxidative damage plays a significant pathological role in many diseases⁽⁸⁾. Oxidative stress is also closely related to gastrointestinal diseases, including intestinal ischemia, radiation enteritis, inflammatory bowel diseases and promotion of gastric and colorectal cancers⁽⁹⁻¹¹⁾. Gastrointestinal tract is an extremely complex ecology. Nutrients including antioxidants through GI are important for maintaining physiology functions and health. Humans and other organisms possess antioxidant defense and repair systems to protect them against oxidative damage; however, these systems are not effective enough to eliminate the damage completely⁽¹²⁾. Therefore, antioxidant supplements or foods containing antioxidants may be used to help the human body reduce oxidative damage.

Yogurt bacteria *S. salivarius* ssp. *thermophilus* ATCC 19258 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 have been used in various fermented dairy products. These two strains were, therefore, used in this study to evaluate their probiotic effects. The objective of this study was to investigate the antioxidative effect of intracellular extracts from yogurt bacteria *S. salivarius* ssp. *thermophilus* ATCC 19258 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 on protecting Intestine 407 cells from the genotoxicity and cytotoxicity of the oxidant H₂O₂.

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MATERIALS AND METHODS

I. Bacterial Strains

Yogurt bacteria *Streptococcus salivarius* ssp. *thermophilus* ATCC 19258 and *Lactobacillus delbrueckii* ssp. *bulgaricus* ATCC 11842 were obtained from our frozen stock culture collection. *S. salivarius* ssp. *thermophilus* ATCC 19258 was grown in M17 (Difco Laboratories, Detroit, Michigan) and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 was grown in MRS (Difco Laboratories, Detroit, Michigan) at 37°C. Both strains were serially transferred at least three times prior to use in these studies.

II. Acid and Bile Tolerance of Yogurt Bacteria

Acid tolerance was determined by growing *S. salivarius* ssp. *thermophilus* ATCC 19258 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 in culture broth adjusted to pH 2 and 4. Bile tolerance was determined by growing *S. salivarius* ssp. *thermophilus* ATCC 19258 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 in culture broth supplemented with 0 and 0.3% oxgall⁽¹³⁻¹⁵⁾ (dehydrated fresh bile; Difco Laboratories, Detroit, Michigan). Cells were inoculated at 2% and incubated at 37°C. Aliquots of *S. salivarius* ssp. *thermophilus* ATCC 19258 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 cultures were taken hourly and the viable cell counts were determined for both acid tolerance and bile tolerance by enumerating on plates following anaerobic incubation (BBL GasPak anaerobic system; Becton Dickinson and Company, Cockeysville, Maryland).

III. Preparation of Intracellular Extracts of Yogurt Bacteria

Cells of *S. salivarius* ssp. *thermophilus* ATCC 19258 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 were harvested by centrifugation at 4400 xg for 10 min after 18 h incubation at 37°C. Cell pellets were quickly washed twice with deionized water. Cells were resuspended in deionized water followed by ultrasonic disruption (Setting = 4; XL-2020 sonicator; Heat System, Farmingdale, New York). Sonication was performed for three 1-min intervals in an ice bath. Cells debris was removed by centrifugation at 7800 xg for 10 min and the resulting supernatant was the intracellular extracts. Total cell numbers were adjusted to 10⁹ CFU/mL (equivalent to a final protein concentration of 1.4 ± 0.3 µg/mL) for the preparation of intracellular extracts.

IV. Antioxidative Activity of Yogurt Bacteria

The measurement of antioxidative activity of *S. salivarius* ssp. *thermophilus* ATCC 19258 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 was performed by the thiobarbituric acid (TBA) method via the measurement of lipid peroxidation⁽¹⁷⁾, based on the monitoring of inhibi-

tion of linoleic acid or human plasma peroxidatin by intracellular extracts of yogurt bacteria.

Linoleic acid was chosen as the unsaturated fatty acid⁽¹⁶⁾. Twenty mL of linoleic acid emulsion was made up of 0.1 mL of linoleic acid (Amersham Life Science, Cleveland, Ohio), 0.2 mL of Tween 20, and 19.7 mL of deionized water. Five tenths mL of phosphate buffer solution (0.02 M, pH = 7.4), 1 mL of linoleic acid emulsion, 0.2 mL of FeSO₄ (0.01%), 0.2 mL of H₂O₂ (0.56 mM), and 0.4 mL of intracellular extract were mixed and incubated at 37°C. Intracellular extract was replaced by deionized water in the control samples. After 12 h of incubation, 2 mL of the reaction solution was mixed with 0.2 mL of trichloroacetic acid (TCA; 4%), 2 mL of TBA (0.8%), and 0.2 mL of butylated hydroxytoluene (BHT; 0.4%). This mixture was incubated at 100°C for 30 min and allowed to cool. Two mL of chloroform was then added for extraction. The extract was obtained and the absorbance was measured at 532 nm. The percentage of inhibition of linoleic acid peroxidatin was defined as follows: [1-A₅₃₂(experimental sample)/A₅₃₂(control)] X 100%.

The plasma lipid peroxidation was analyzed by the method developed by Rodriduez and Ruiz⁽¹⁸⁾. Four tenths mL of plasma (Blood Center, Taichung, Taiwan), 0.1 mL of FeSO₄ solution (50 µM), and 0.2 mL of intracellular extract were mixed and incubated at 37°C in a water bath. Intracellular extract was replaced by deionized water in the control samples. After 12 h of incubation, the reaction solution was mixed with 0.375 mL of 4% TCA and 75 µM of BHT (0.5 mM) and placed in an ice bath for 5 min. The upper phase was obtained by centrifugation at 3000 xg for 10 min. Two tenths mL of TBA (0.6%) was then added. This mixture was incubated at 100°C for 30 min and allowed to cool. The absorbance was then measured at 532 nm. The percentage of inhibition of plasma lipid peroxidation was also defined as [1-A₅₃₂(experimental sample)/A₅₃₂(control)] X 100%.

V. Cell Culture

Intestine 407 (CCRC 60022) cells were purchased from the Culture Collection and Research Center (CCRC; Hsinchu, Taiwan). Cells were grown in basal medium eagle (BME) supplemented with Earle's BSS and 15% fetal bovine serum (Gibco BRL, Life technologies, New York) and incubated in a humidified atmosphere of air/CO₂ (95:5 v/v) at 37°C. Cells were grown from 10⁴⁻⁵ to 10⁵⁻⁶ cells/mL in 2 to 3 days. Confluent cells were washed twice with PBS. One mL of trypsin-EDTA (0.05% trypsin and 0.53 mM EDTA) was added and left for 5 min. Two mL of BME was then added and cells were harvested by centrifugation at 1000 xg for 5 min.

VI. Comet Assay

Comet assay (single cell microgel electrophoresis) was used for measuring DNA damage by the oxidant,

H₂O₂. The comet assay, based on the method of Singh *et al.*⁽¹⁹⁾, was carried out as described by Gedik *et al.*⁽²⁰⁾ and Collins *et al.*⁽²¹⁾. One mL of Intestine 407 cells (2 X 10⁵ cells) was treated with 250 µL of H₂O₂ (0.8 mM) and one mL of intracellular extract of 10⁹ yogurt bacteria cells for 1 h. Hydrogen peroxide and intracellular extract were replaced with deionized water in the blank samples. Each microscope slide was covered with 100 µL of 0.5% normal melting point agarose in PBS at 45°C. They were immediately covered with a cover slip and then kept at 4°C until the agarose had solidified. Seventy-five microliter of 1% low melting point agarose at 37°C was added to the treated Intestine 407 cells (1 X 10⁵ cells) suspended in 10 µL of PBS. After removing the cover slip, the cell suspension was rapidly pipetted on to the first agarose layer, spread using a cover slip, and allowed to solidify at 4°C. A final layer of 75 µL of 1% low melting point agarose was applied in the same way. The slides were immersed in lysis solution (100 mM Na₂EDTA, 1% Triton X-100, 2.5 M NaCl, 1% lauryl, 10 mM Tris, 10% dimethyl sulfoxide; freshly prepared) for 1 h. Afterwards, slides were removed from the lysis solution, drained and placed in a horizontal gel electrophoresis tank. Electrophoresis was conducted for 20 min at 20 V adjusted to 300 mA. Slides were then drained, placed on a tray and flooded slowly with Tris buffer (0.4 M, pH 7.5) for 5 min. The slides were again drained before being stained with 50 µL of ethidium bromide (20 µg/mL). Slides were stored in a closed container at 4°C and analyzed within 24 h. Analysis was performed immediately after staining, using a 200 X objective with a optiphot microscope equipped with an excitation filter of 515-560 nm from a 100-W mercury lamp and a barrier filter of 590 nm. Comets form as broken ends of the negatively charged DNA molecule becomes free to migrate in the electric field. One hundred cells were analyzed for each sample population and cells were categorized as undamaged DNA, minimal DNA damage and extensive DNA damage (Figure 1). Minimal DNA damage is defined as DNA at low damage levels stretches of attached strands of DNA. Extensive DNA damage is defined when DNA has increasing numbers of breaks, DNA pieces migrate freely into the tail forming comet images.

VII. MTT Colorimetric Assay

The inhibition of oxidant H₂O₂ cytotoxicity to Intestine 407 cells by *S. salivarius* ssp. *thermophilus* ATCC 19258 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 was determined by the MTT colorimetric assay⁽²²⁻²⁵⁾. 3-(4,5-Dimethylthiazol-2-yl) -2,5-diphenol tetrazolium bromide (MTT; Sigma, St. Louis, Missouri) was used as a tetrazolium salt. One mL of BME was inoculated with 5 X 10⁴ Intestine 407 viable cells and incubated under the conditions described previously for 16 to 18 h. One twentieth mL of H₂O₂ (8 mM) was added as an oxidant. One mL of intracellular extract of yogurt bacteria (10⁹ cells)

was then added. Hydrogen peroxide and intracellular extract were replaced with deionized water in the control samples. After 24 h of reaction at 37°C, the solution was mixed with 1 mL of MTT (2 mg/mL) and incubated at 37°C for 2.5 h. One mL of dimethyl sulfoxide was then added to dissolve the blue crystals and absorbance was read at 492 nm. The percentage of cytotoxicity inhibition was defined as follows: $\{[A_{492}(\text{sample treated with H}_2\text{O}_2 \text{ and intracellular extract}) - A_{492}(\text{sample treated with H}_2\text{O}_2)] / [A_{492}(\text{blank}) - A_{492}(\text{sample treated with H}_2\text{O}_2)]\} \times 100\%$.

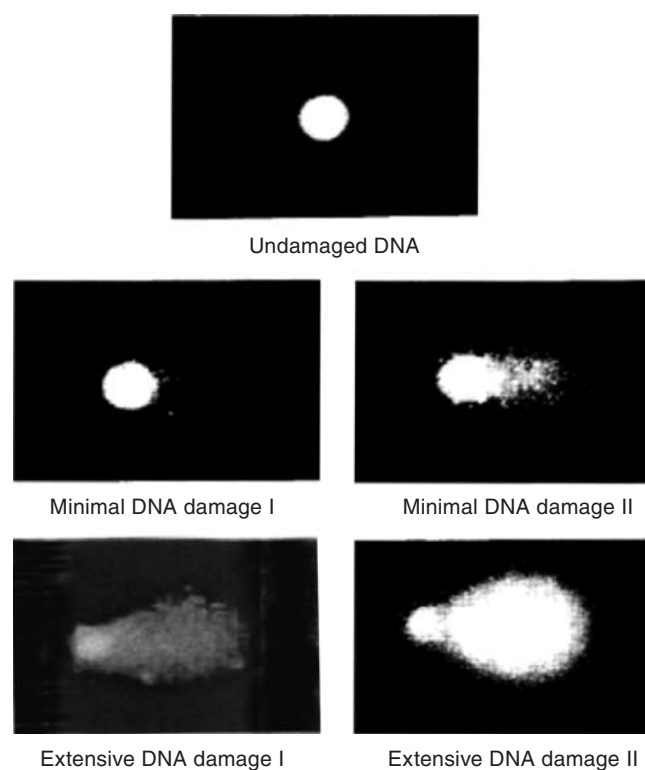


Figure 1. Grade of DNA damage in comet assay (■ 20µm)

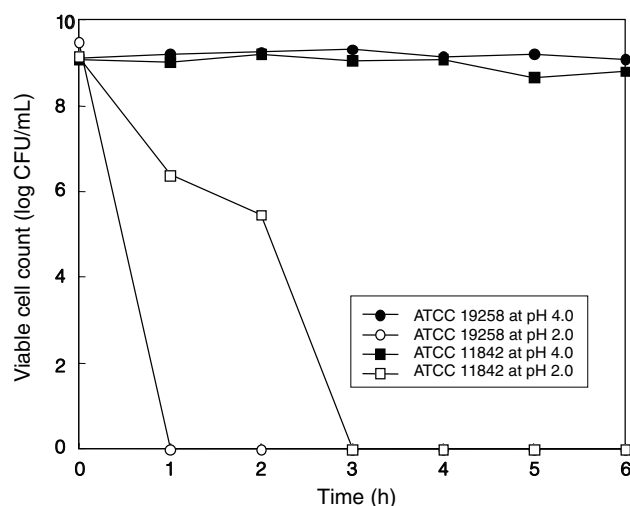


Figure 2. Acid toletance of *S. salivarius* ssp. *thermophilus* ATCC 19258 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842

VIII. Statistical Analysis

Data reported in tables were the means of triplicate experiments. The least significant difference test was used to compare means⁽²⁶⁾.

RESULTS AND DISCUSSION

The acid tolerance and bile tolerance of yogurt bacteria used in this study are illustrated in Figures 2 and 3, respectively. Yogurt bacteria were tested for their ability to survive conditions mimic those present in the gastrointestinal tract. The fermented yogurt products usually have pH around 4.2 to 4.5. As shown in Figure 2, both yogurt strains survived well at pH 4, indicating that yogurt bacteria would stay alive at the pH values of the fermented products. However, yogurt bacteria were unable to survive at pH 2, which is the pH value of gastric acid. Although a small fraction of *S. salivarius* ssp. *thermophilus* ATCC 19258 cells tolerated bile (Figure 3), this strain was not able to survive at the pH of gastric acid. *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 was more acid tolerant than *S. salivarius* ssp. *thermophilus* ATCC 19258 at pH 2, but it was highly bile sensitive. Results from the acid tolerance study and the bile tolerance study both indicated that most cells of these two yogurt bacteria would release their intracellular constituents in the GI tract. Therefore, the intracellular extracts of yogurt bacteria were evaluated for their antioxidative effects.

Inhibition of lipid peroxidation is commonly used for analysis of antioxidative activity. Antioxidative activity of yogurt bacteria was measured based on the inhibition of lipid peroxidation of two model systems, including a free fatty acid (linoleic acid) system and a biological lipid (plasma lipid) system. The inhibitory rate on linoleic acid peroxidation was 61 and 57% for *S. salivarius* ssp. *thermophilus* ATCC 19258 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842, respectively (Table 1). The inhibitory rate on plasma lipid peroxidation was 57 and 41% for *S. salivarius* ssp. *thermophilus* ATCC 19258 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842, respectively (Table 2). In our previous study⁽³²⁾, two intestinal lactic acid bacteria *Bifidobacterium longum* ATCC 15708 and *Lactobacillus acidophilus* ATCC 4356 were also found antioxidative. Intracellular extracts from *B. longum* ATCC 15708 and *L. acidophilus* ATCC 4356 demonstrating the inhibitory rate on linoleic acid peroxidation was 48 and 45%, respectively. The inhibitory rate on plasma lipid peroxidation was 34 and 29 for *B. longum* ATCC 15708 and *L. acidophilus* ATCC 4356, respectively. Results from both systems demonstrated that these two yogurt strains were highly antioxidative. Further experiments were done on Intestine 407 cells to determine the antioxidative effects of yogurt bacteria on reducing oxidative damage of oxidant H₂O₂ (Table 3).

Oxidative stress can damage biological molecules,

e.g., DNA, proteins, and lipids. DNA is usually a more significant target of injury than are lipids although lipid peroxidation is usually used for the analysis of antioxidative activity. DNA damage often occurs before lipid peroxidation actually happens⁽⁷⁾. Comet assay is becoming a popular tool for measuring DNA strand breaks caused by various chemical agents due to its simplicity

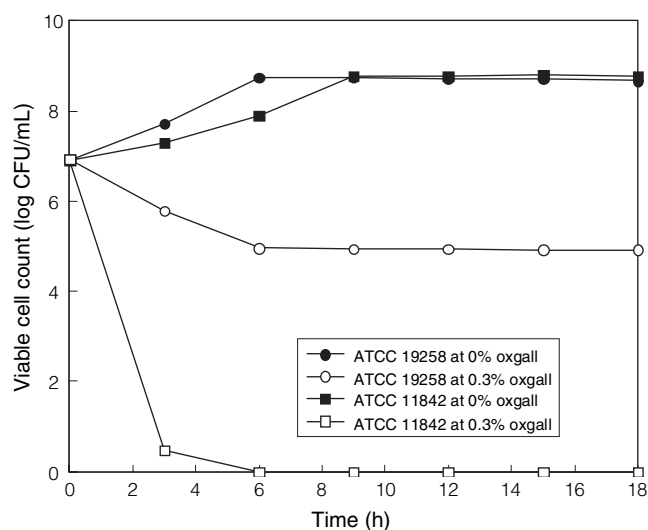


Figure 3. Bile tolerance of *S. salivarius* ssp. *thermophilus* ATCC 19258 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842

Table 1. Inhibition of linoleic acid peroxidation by two yogurt bacteria

Strain	Inhibition (%) ^{1,2}
<i>S. salivarius</i> ssp. <i>thermophilus</i> ATCC 19258	61.4 ± 3.2 ^a
<i>L. delbrueckii</i> ssp. <i>bulgaricus</i> ATCC 11842	56.9 ± 3.7 ^a

¹Inhibition of linoleic acid peroxidation by intracellular extract of 10⁹ cells of yogurt bacteria. The percentage of inhibition of linoleic acid peroxidation was defined as follows: [1-A₅₃₂(experimental sample)/A₅₃₂(control)] X 100%.

²Least significant difference pairwise comparisons. Values in the same column with different letter superscripts are significantly different (P < 0.05). Data were means ± SD of triplicate experiments.

Table 2. Inhibition of plasma lipid peroxidation by two yogurt bacteria

Strain	Inhibition (%) ^{1,2}
<i>S. salivarius</i> ssp. <i>thermophilus</i> ATCC 19258	57.2 ± 1.8 ^a
<i>L. delbrueckii</i> ssp. <i>bulgaricus</i> ATCC 11842	40.6 ± 3.4 ^b

¹Inhibition of linoleic acid peroxidation by intracellular extract of 10⁹ cells of yogurt bacteria. The percentage of inhibition of linoleic acid peroxidation was defined as follows: [1-A₅₃₂(experimental sample)/A₅₃₂(control)] X 100%.

²Least significant difference pairwise comparisons. Values in the same column with different letter superscripts are significantly different (P < 0.05). Data were means ± SD of triplicate experiments.

Table 3. Inhibition of H₂O₂ genotoxicity to Intestine 407 cells by *S. salivarius* ssp. *thermophilus* ATCC 19258 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842

Sample	Grade of DNA damage in 100 Intestine 407 cells		
	Undamaged DNA	Minimal DNA damage	Extensive DNA damage
Control	100	0	0
+ H ₂ O ₂ only	0	0	100
+ H ₂ O ₂ and ATCC 19258	52	43	5
+ H ₂ O ₂ and ATCC 11842	0	71	29

¹Genotoxicity inhibition by intracellular extract of 10⁹ cells of yogurt bacteria.

²Data were means of triplicate experiments.

Table 4. Inhibition of H₂O₂ cytotoxicity to Intestine 407 cells by *S. salivarius* ssp. *thermophilus* ATCC 19258 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842

Strain	Inhibition (%) ^{1, 2}
<i>S. salivarius</i> ssp. <i>thermophilus</i> ATCC 19258	70.8 ± 4.7 ^a
<i>L. delbrueckii</i> ssp. <i>bulgaricus</i> ATCC 11842	48.2 ± 3.9 ^b

¹Cytotoxicity inhibition by intracellular extract of 10⁹ cells of yogurt bacteria. The percentage of cytotoxicity inhibition was defined as follows: {[A₄₉₂(sample treated with H₂O₂ and intracellular extract) - A₄₉₂(sample treated with H₂O₂)/A₄₉₂(control) - A₄₉₂(sample treated with H₂O₂)] X 100%.

²Least significant difference pairwise comparisons. Values in the same column with different letter superscripts are significantly different (P < 0.05). Data were means ± SD of triplicate experiments.

and rapidity⁽²⁷⁻²⁸⁾. The comet assay was used in this study for determining the antioxidative effect of yogurt bacteria on protecting Intestine 407 cells from the genotoxicity of oxidant H₂O₂. The longer tails (extensive migration) from the microgel electrophoresis indicated that DNA was damaged more extensively. The final concentration of H₂O₂ used in the comet assay was 89 μM, the concentration that caused extensive DNA damage (data not shown). As shown in Table 3, intracellular extracts of yogurt bacteria reduced the genotoxicity of H₂O₂. Although *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 had only minor effects on reducing DNA damage, *S. salivarius* ssp. *thermophilus* ATCC 19258 demonstrated excellent potential for protecting the Intestine 407 cells from the genotoxicity of oxidant H₂O₂.

Oxidative damage can cause the death of cells when the damage is too serious to repair⁽⁷⁾. Intestine 407 cells were also used to evaluate the antioxidative effect of these two yogurt strains on the inhibition of H₂O₂ cytotoxicity by the MTT colorimetric assay. In MTT colorimetric assay, we used 0.19 mM (final concentration) H₂O₂, which caused approximately 55% cell death (data not shown). The cytotoxicity of H₂O₂ to Intestine 407 cells was

reduced by *S. salivarius* ssp. *thermophilus* ATCC 19258 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 as shown in Table 4. The cytotoxicity inhibition rate was 71 and 48% for *S. salivarius* ssp. *thermophilus* ATCC 19258 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842, respectively. Both yogurt bacteria showed high cytotoxicity inhibitory ability for oxidant H₂O₂.

In this study, the antioxidative effects were found for the intracellular extracts of yogurt bacteria in reducing genotoxicity and cytotoxicity *in vitro*. According to our previous study⁽⁵⁾, the intracellular extracts of lactic acid bacteria have metal ion chelating ability, reactive oxygen species scavenging ability and reduction activity. Although conditions in the GI tract are very complicated, the study of Kaizu *et al.*⁽³⁾ demonstrated that the intracellular extract is also antioxidative *in vivo*. In addition, Kaizu *et al.* have demonstrated that hemolysis of red blood cells was inhibited in rats which were administered the intracellular extracts of *Lactobacillus* sp. SBT 2028. Rats which were deficient in vitamin E, a well-known natural antioxidant, were used for the experiments. The results provided evidence that the intracellular extract is antioxidative and, therefore, improved the vitamin E deficiency status. It is reasonable to expect a significant number of yogurt bacteria lyse, which release their intracellular antioxidative constituents during transit through the GI tract⁽²⁹⁻³⁰⁾. The results of this study demonstrated the antioxidative potential of yogurt bacteria *S. salivarius* ssp. *thermophilus* ATCC 19258 and *L. delbrueckii* ssp. *bulgaricus* ATCC 11842 for protecting Intestine 407 cells from the genotoxicity and cytotoxicity of oxidants. We strongly recommend that consumers eat yogurt or other lactic acid bacteria fermented products containing live probiotic bacteria. However, these probiotic bacteria do not necessarily have to be acid or bile resistant to stay alive in gastrointestinal tract to be probiotic, i.e. antioxidative in this study. Intact cells of lactic acid bacteria were indeed found possessing antioxidative ability *in vitro*⁽³²⁾. Nevertheless, using the intact cells as the delivery vehicles passing through the gastrointestinal tract, the intracellular constituents released from the lactic acid bacteria in GI tract can be also antioxidative. Consumption of yogurt bacteria and other lactic acid bacteria containing foods may be recommended as healthy. It is well established that a wide variety of oxygen-centered free radicals and other reactive oxygen species are continuously produced in the human body and in food systems⁽⁷⁾. Antioxidants are needed for practical applications. Although various synthetic and natural antioxidants have been reported, the safety and long-term effects on health of synthetic antioxidants are to be confirmed. Antioxidants from natural sources are likely to be more desirable. Besides the long history of consumption, which proves the safety of consuming yogurt bacteria, they have been reported to have health-promoting characteristics that make these microorganisms desirable for use in the production of various health and functional foods. Interest in the role of

yogurt bacteria and other lactic acid bacteria in promoting human health dates at least as far as 1908 when Metchnikoff suggested that consumption of milk fermented with lactobacilli would prolong life⁽³¹⁾. If this is true, longevity may be partially due to the antioxidative ability of lactic acid bacteria. The results of this study suggest that yogurt bacteria are potential candidates for production of functional foods and of natural antioxidant supplements that help to promote longevity and a healthy life.

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