Antioxidative Ability of Lactic Acid Bacteria

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Nineteen strains of lactic acid bacteria were investigated for antioxidative activity. These included *Lactobacillus acidophilus* B, E, N1, 4356, LA-1, and Farr; *Lactobacillus bulgaricus* 12 278, 448, 449, Lb, 1006, and 11 842; *Streptococcus thermophilus* 821, MC, 573, 3641, and 19 987; and *Bifidobacterium longum* B6 and 15 708. Intracellular cell-free extract of all strains demonstrated antioxidative activity with inhibition rates of ascorbate autoxidation in the range of 7−12%. Antioxidative mechanisms including metal ion chelating ability, scavenge of reactive oxygen species, enzyme inhibition, and reducing activity of intracellular cell-free extract of lactic acid bacteria were studied. *S. thermophilus* 821 had the highest metal ion chelating ability for Fe^{2+}, and *B. longum* 15 708 showed the highest Cu^{2+} chelating ability among the 19 strains tested. All strains demonstrated reactive oxygen species scavenging ability. *L. acidophilus* E showed the highest hydroxyl radical scavenging ability, and *B. longum* B6 had the best hydrogen peroxide scavenging ability. Reducing activity was also found in all strains. Most of the strains tested demonstrated excellent reducing activity. *B. longum* B6 showed the highest reducing activity among the 19 strains tested. In enzyme inhibition, superoxide dismutase activity was not found in these 19 strains, and the activity of superoxide dismutase was not induced when metal ion Mn^{2+}, Fe^{2+}, or $Cu^{2+}Zn^{2+}$ was present.

Keywords: *Lactic acid bacteria; oxidation; antioxidative activity; antioxidative mechnisms*

INTRODUCTION

Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, oxidative stress can also damage biological molecules. It is well established that oxygen-centered free radicals and other reactive oxygen species are continuously produced in vivo (Halliwell and Chirico, 1993). The term reactive oxygen species is used by investigators to include both oxygen-centered radicals and nonradical derivatives of oxygen. A wide variety of reactive oxygen species can be formed in the human body and in food systems. Oxidative damage plays a significant pathological role in human diseases. Cancer, emphysema, cirrhosis, atherosclerosis, and arthritis have all been correlated with oxidative damage (Halliwell and Gutteridge, 1984). Although humans and other organisms possess antioxidant defense and repair systems which have evolved to protect them against oxidative damage, these systems are not effective enough to totally prevent the damage (Simic, 1988). However, antioxidant supplements or foods containing antioxidants may be used to help the human body reduce oxidative damage.

Various synthetic and natural antioxidants have been reported; however, there are doubts about the safety and long-term effects on health of synthetic antioxidants. Antioxidants from natural sources are likely to be more desirable. The effect of antioxidative lactic acid bacteria on rats deficient in vitamin E has been studied by Kaizu et al. (1993). Also, Sanders et al. (1995) reported that *Lactococcus* demonstrates antioxidative superoxide dismutase enzyme activity. Besides the long history of consumption which proves the safety of consuming lactic acid bacteria, lactic cultures have been reported to have health-promoting characteristics that make these microorganisms desirable for use in the production of dairy and other food products (Fernandes and Shahani, 1989). Interest in the role of lactic acid bacteria in promoting human health goes back at least as far as 1908 when Metchnikoff suggested that consumption of milk fermented with lactobacilli would prolong life (Metchnikoff, 1908). If this is true, the longevity may be, in part, due to the antioxidative ability of lactic acid bacteria. However, there is not much data about the antioxidative ability of lactic cultures available to date.

The objectives of this study were to investigate the antioxidative activity of lactic acid bacteria and to identify the mechanisms of antioxidation.

MATERIALS AND METHODS

Bacterial Strains. Nineteen strains of lactic acid bacteria were studied. These included *Lactobacillus acidophilus* B, E, N1, 4356, LA-1, and Farr; *Lactobacillus bulgaricus* 12 278, 448, 449, Lb, 1006, and 11 842; *Streptococcus thermophilus* 821, MC, 573, 3641, and 19 987; and *Bifidobacterium longum* B6 and 15 708. All these strains were obtained from our frozen stock culture collection and grown at 37 °C in modified MRS, in which magnesium sulfate, manganese sulfate, and dipotassium phosphate were eliminated from the formula. Agar plates were made by adding 1.5% agar (Difco Laboratories, Detroit, MI) to broth for viable cell counts and were incubated at 37 °C for 48 h anaerobically (BBL GasPak anaerobic system; Becton Dickinson and Company, Cockeysville, MI). All strains were serially transferred at least three times prior to use in studies.

Preparation of Intracellular Cell-Free Extract of Lactic Acid Bacteria. Cells of 19 strains of lactic acid

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bacteria were harvested by centrifugation at 6000 rpm for 10 min after 18 h of incubation at 37 °C. Cell pallets were then quickly washed twice with deionized water and resuspended in deionized water followed by ultrasonic disruption (setting = 4; Sonicator XL-2020; Heat System, Farmingdale, NY). Sonication was performed for five 1 min intervals in ice bath. Cell debris was removed by centrifugation at 8000 rpm for 10 min, and the resulting supernatant was the intracellular cell-free extract. Total cell numbers were adjusted to 10^{10} cfu/mL prior to preparation of cell-free extracts.

Antioxidative Activity of Lactic Acid Bacteria. The antioxidative activity of 19 strains of lactic acid bacteria were assayed by the method of inhibition of ascorbate autoxidation (Mishra and Korachich, 1984). One-tenth of the intracellular cell-free extract was added to 0.1 mL of 5 mM ascorbate stock solution and 9.8 mL of 0.2 M phosphate buffer (pH 7); the mixture was then quickly transferred to a cuvette for absorbance reading at 265 nm (A_{265}) for 10 min at 37 °C. Percentage of inhibition of ascorbate autoxidation was defined as follows: $[1 - A_{265}(\text{sample})/A_{265}(\text{blank})] \times 100\%$.

Metal Ion Chelating Assay. The chelating ability of intracellular cell-free extract of lactic acid bacteria for ferrous and cupric ions was determined.

For ferrous ion chelating ability, the method developed by Yamauchi et al. (1984) was modified for use; 0.5 mL of intracellular cell-free extract was mixed with 0.1 mL of ascorbate (1 g/dL), 0.1 mL of $FeSO_4$ (0.4 g/dL), and 1 mL of NaOH (0.2 M). The mixture was incubated at 37 °C in water bath; 0.2 mL of trichloroacetic acid (TCA; 10%) was added following incubation for 20 min. Supernatant was obtained by centrifugation at 3000 rpm for 10 min, and 0.5 mL of *o*-phenanthroline (1 g/L) was added. After 10 min of reaction, the absorbance was measured at 510 nm.

Cupric ion chelating analysis was performed as described by Looyenga and Boltz (1971). Intracellular cell-free extract (0.1 mL), 0.1 mL of $CuSO_4$ (100 μM), 0.2 mL of HCl (0.02 M), and 0.1 mL of HOCl (0.5 g/dL) were mixed and incubated at 37 °C for 20 min; 0.2 mL of pyrrolidinedithiocarbamate ammonium salt (APDTC; 0.1 g/dL) was added and allowed to stand for 5 min. Two milliliters of chloroform was added and the mixture was shaken vigorously for 1 min. The extract was obtained and absorbance was measured at 269 nm.

Scavenge of Reactive Oxygen Species. Scavenging ability of intracellular cell-free extract of lactic acid bacteria for hydroxyl radical and hydrogen peroxide was analyzed.

To determine the hydroxyl radical scavenging ability, hydroxyl was first generated by ultraviolet radiation (Tabatabaie and Floyd, 1994) and then detected by using a modification of the method of Gutteridge (1987). Hydroxyl was generated by ultraviolet radiation of 1 mL of deionized water using an ultraviolet lamp (265 nm; model 206; Chousin, Taipei, Taiwan) for 25 min. Five-tenths of a milliliter of intracellular cell-free extract was added and the mixture was allowed to stand for 3 min. Then, 0.5 mL of benzoic acid (10^{-4} M) was added, and the mixture was allowed to react for 1 min; a fluorescent spectrophotometer (model 650-40; Hitachi, Tokyo, Japan) was then used for measurement (Ex = 305 and Em = 407). The hydroxyl radical scavenging ability of uric acid was used as the standard. The same methods described above were used to analyze the hydroxyl scavenging ability of uric acid except that uric acid was a replacement for intracellular cell-free extract.

The scavenge of hydrogen peroxide was determined by Wolfe's method (1962). Five-tenths of a milliliter of intracellular cell-free extract and 0.1 mL of hydrogen peroxide (2×10^{-3} M) were mixed and incubated at 25 °C for 20 min in the dark. Then, 0.2 mL of TiCl (10 mg/mL) was added and left for 1 min. The upper phase was obtained by centrifugation at 8000 rpm for 3 min. The scavenged hydrogen peroxide was monitored by measuring absorbance decrease at 412 nm.

Reducing Activity of Lactic Acid Bacteria. The method developed by Oyaizu (1986) was used to evaluate the reducing activity of lactic acid bacteria. Five-tenths of a milliliter of potassium ferricyanide (1%) was mixed with the same volume of phosphate buffer (0.02 M) and intracellular cell-free extract. The reaction mixture was incubated at 50 °C for 20 min, then cooled rapidly, and 0.5 mL of TCA (10%) was added. One and one-half milliliters of upper phase was obtained by centrifugation at 3000 rpm for 5 min, and 0.2 mL of $FeCl_3$ (0.1%) was added. Absorbance was read at 700 nm. Cysteine was used as the standard for expression of reducing activity.

Superoxide Dismutase (SOD) Activity and SOD Induction. SOD determination was performed by the method of Kim et al. (1991). Three-tenths of a milliliter of intracellular cell-free extract, 1 mL of EDTA (1 mM), 0.3 mL of epinephrine (0.3 mM, pH 2), and 2 mL of sodium carbonate (0.05 M) were mixed together. The mixture was transferred to a cuvette for absorbance reading at 480 nm for 3 min. (SOD activity of the intracellular cell-free extract was calculated from the standard curve of purified bovine erythrocyte SOD).

Two methods were used to induce SOD activity of lactic acid bacteria. Method 1: Lactic acid bacteria were incubated in modified MRS at 37 °C for 18 h. Cells were harvested by centrifugation at 6000 rpm for 3 min. Cells were then shaken for 3 h in MRS containing 0.01% metal ion Mn^{2+}, Fe^{2+}, or Cu^{2+}-Zn^{2+}. $MnSO_4$, $FeSO_4$, $CuSO_4$, and $ZnSO_4$ were used as the sources of metal ions. Method 2: Lactic acid bacteria were grown in modified MRS plus 0.01% metal ion Mn^{2+}, Fe^{2+}, or $Cu^{2+}Zn^{2+}$ at 37 °C for 18 h. Intracellular cell-free extract was prepared from the culture and the SOD activity was analyzed.

Statistical Analysis. Data reported in tables were the means of experiments repeated three times. The least significant difference test was used to compare means (Steel and Torrie, 1980).

RESULTS

Growth in Modified MRS. All 19 lactic acid bacterial strains were grown well in modified MRS (data not shown). Most of the strains were grown to 10^8 cfu/mL except for *L. acidophilus* B, E, N1, and 4356 which were grown to 10^9 cfu/mL, and *S. thermophilus* 19987 which was grown to 10^{10} cfu/mL.

Antioxidative Activity of Lactic Acid Bacteria. The results of antioxidative activity of intracellular cell-free extract of 19 strains of lactic acid bacteria grown in the modified MRS without any added metal ion cofactors are shown in Table 1. All 19 strains tested demonstrated the antioxidative activity with inhibition rate in the range 7.2−12.3%. Among the 19 strains, *L. bulgaricus* 448, 449, 1006, *S. thermophilus* 821, and *B. longum* B6, 15708 showed higher inhibition rate in the range 11.0−12.3%. However, no particular genus or species demonstrated obvious higher antioxidative activity than others among *L. acidophilus*, *L. bulgaricus*, *S. thermophilus*, and *B. longum*. At the first stage of screening for antioxidative activity, all 19 strains demonstrated ability to inhibit oxidation.

Metal Ion Chelating Ability. The results of chelating ability of intracellular cell-free extract of lactic acid bacteria for Fe^{2+} and Cu^{2+} are listed in Table 2. The results were calculated based on the same cell number at 1×10^{10}. *L. bulgaricus* strains, generally speaking, demonstrated higher metal ion Fe^{2+} chelating ability (ranging 5.6−52.9 ppm) than *L. acidophilus*. *S. thermophilus* strains showed a very wide range of Fe^{2+} chelating ability ranging 0.5−72.7 ppm. *S. thermophilus* 821 and 19 987 demonstrated the highest and lowest Fe^{2+} chelating ability, respectively, among the 19 strains of lactic acid bacteria tested. *B. longum* B6 and 15708 had high chelating activity for Fe^{2+} at 40.7 and 26.6 ppm, respectively.

For metal ion Cu^{2+} chelating, *L. acidophilus* and *S. thermophilus* strains showed a wide range of ability ranging from 0 to 34.8 ppm and from 2.4 to 51.0 ppm,

Table 1. Inhibition of Ascorbate Autoxidation by Intracellular Cell-Free Extract of Lactic Acid Bacteria

strain	inhibition (%)[a,b]
Lactobacillus acidophilus	
B	9.6[c]
E	10.3[b]
N1	7.4[d]
4356	8.6[c]
LA-1	9.8[b,c]
Farr	10.3[b]
Lactobacillus bulgaricus	
12 278	9.3[c]
448	11.0[a]
449	11.4[a]
Lb	8.1[d]
1006	12.3[a]
11 842	10.1[b]
Streptococcus thermophilus	
821	12.0[a]
MC	10.6[b]
573	8.8[c]
3641	9.6[c]
19 987	7.2[d]
Bifidobacterium longum	
B6	11.4[a]
15 708	11.8[a]

[a] Percentage of inhibition of ascorbate autoxidation was defined as $[1 - A_{265}(\text{sample})/A_{265}(\text{blank})] \times 100(\%)$. [b] Least significant difference pairwise comparisons. Values in the same column with different letter superscripts are significantly different ($P < 0.05$).

Table 2. Chelating Ability of Intracellular Cell-Free Extract of Lactic Acid Bacteria

	concentration of chelated metal ion (ppm)[a,b]	
strain	Fe(II)	Cu(II)
Lactobacillus acidophilus		
B	2.5[e]	0.0[f]
E	6.6[d]	0.9[f]
N1	5.1[d]	5.7[e]
4356	7.0[d]	3.7[e,f]
LA-1	2.3[e]	34.8[c]
Farr	6.0[d]	12.5[e]
Lactobacillus bulgaricus		
12 278	5.6[d]	45.5[b]
448	5.7[d]	48.3[b]
449	52.9[b]	13.3[e]
Lb	13.1[c,d]	23.9[d]
1006	23.3[c]	32.1[c]
11 842	11.3[c,d]	26.8[c,d]
Streptococcus thermophilus		
821	72.7[a]	51.0[b]
MC	2.9[e]	38.9[c]
573	22.8[c]	21.6[d]
3641	42.8[b]	3.8[e,f]
19 987	0.5[e]	2.4[e,f]
Bifidobacterium longum		
B6	40.7[b]	18.5[d]
15 708	26.6[c]	63.3[a]

[a] The chelating ability of intracellular cell-free extract of 10^{10} viable lactic acid bacterial cells. [b] Least significant difference pairwise comparisons. Values in the same column with different letter superscripts are significantly different ($P < 0.05$).

respectively. Six *L. bulgaricus* strains tested had high Cu^{2+} chelating ability ranging from 13.2 to 48.3 ppm. Both *B. longum* B6 and 15 708 had not only high Fe^{2+} chelating ability but also high Cu^{2+} chelating ability (at 18.5 and 63 ppm, respectively). Among 19 strains of lactic acid bacteria tested, *L. acidophilus* B showed no Cu^{2+} chelating ability at all and *B. longum* 15708 demonstrated the highest Cu^{2+} chelating ability.

Table 3. Scavenge of Hydroxyl Radical by Intracellular Cell-Free Extract of Lactic Acid Bacteria

strain	equivalent uric acid (mM)[a,b]
Lactobacillus acidophilus	
B	1.4[d]
E	33.1[a]
N1	29.5[a]
4356	5.1[c,d]
LA-1	3.5[c,d]
Farr	2.8[d]
Lactobacillus bulgaricus	
12 278	11.9[c]
448	15.9[b]
449	11.4[c]
Lb	23.4[a,b]
1006	16.5[b]
11 842	18.2[b]
Streptococcus thermophilus	
821	18.6[b]
MC	8.3[c,d]
573	13.8[b,c]
3641	0.5[d]
19 987	19.8[b]
Bifidobacterium longum	
B6	16.0[b]
15 708	25.5[a]

[a] The hydroxyl radical scavenging ability of intracellular cell-free extract of 10^{10} viable lactic acid bacterial cells is expressed as the equivalent amount of uric acid with the same scavenging ability. [b] Least significant difference pairwise comparisons. Values in the same column with different letter superscripts are significantly different ($P < 0.05$).

Scavenge of Reactive Oxygen Species. The results of hydroxyl radical scavenging by intracellular cell-free extract of lactic acid bacteria are shown in Table 3. The capability of cell-free extract of 10^{10} viable lactic acid bacterial cells for scavenging hydroxyl radical is expressed as the equivalent amount of uric acid, a compound that scavenges hydroxyl radical, with the same scavenging ability. As shown in Table 3, 19 strains demonstrated various levels of ability ranging from as little as 0.5 mM (*S. thermophilus* 3641) to 33.1 mM (*L. acidophilus* E). Generally speaking, strains of *L. acidophilus* and *S. thermophilus* showed a wide range of ability (1.4−33.1 and 0.5−19.8 mM, respectively) and strains of *L. bulgaricus* and *B. longum* had consistently higher activity (11.4−23.4 mM and 16−25.5 mM, respectively).

Table 4 lists the results of hydrogen peroxide scavenged by intracellular cell-free extract of 10^{10} viable lactic acid bacterial cells. Among the 19 strains tested, *B. longum* B6 had the highest hydrogen peroxide scavenging ability at 6.7 mM and *S. thermophilus* 19 987 showed the lowest ability at 0.1 mM. For six *L. acidophilus* strains, LA-1 and Farr showed high hydrogen peroxide scavenging ability, but B, E, N1, and 4356 all had ability lower than 1 mM. Six *L. bulgaricus* and two *B. longum* strains all demonstrated high hydrogen peroxide scavenging ability (>3.1 mM and >4.8 mM, respectively). *S. thermophilus* strains also scavenged hydrogen peroxide well (>2.6 mM) except for strain 19 987 (0.1 mM).

Reducing Activity. Table 5 shows the reducing activity of intracellular cell-free extract of lactic acid bacteria as measured. The reducing activity of 10^{10} viable cells was expressed as an equivalent amount of cysteine. Nineteen strains tested showed various degrees of reducing activity. The reducing activity of *B.*

Table 4. Scavenge of Hydrogen Peroxide by Intracellular Cell-Free Extract of Lactic Acid Bacteria

strain	removed H_2O_2 (mM)[a,b]
Lactobacillus acidophilus	
B	0.6[e]
E	0.4[e]
N1	1.0[e]
4356	0.6[e]
LA-1	3.7[cd]
Farr	5.8[b]
Lactobacillus bulgaricus	
12 278	3.5[d]
448	5.5[b]
449	5.2[b]
Lb	3.1[d]
1006	4.0[c]
11 842	4.0[c]
Streptococcus thermophilus	
821	5.3[b]
MC	5.0[b]
573	3.2[d]
3641	2.7[d]
19 987	0.1[e]
Bifidobacterium longum	
B6	6.7[a]
15 708	4.8[bc]

[a] Concentration of hydrogen peroxide scavenged by intracellular cell-free extract of 10^{10} viable lactic acid bacterial cells. [a] Least significant difference pairwise comparisons. Values in the same column with different letter superscripts are significantly different ($P < 0.05$).

Table 5. Reducing Activity of Intracellular Cell-Free Extract of Lactic Acid Bacteria

strain	equivalent cysteine (μM)[a,b]
Lactobacillus acidophilus	
B	16.2[f]
E	13.0[f]
N1	28.0[e]
4356	18.9[f]
LA-1	103.6[c]
Farr	154.1[b]
Lactobacillus bulgaricus	
12 278	91.7[c]
448	137.2[b]
449	135.8[b]
Lb	94.1[c]
1006	146.8[b]
11 842	105.3[c]
Streptococcus thermophilus	
821	178.8[b]
MC	144.3[b]
573	100.2[c]
3641	72.9[d]
19 987	4.3[f]
Bifidobacterium longum	
B6	225.3[a]
15 708	152.5[b]

[a] The reducing activity of 10^{10} viable lactic acid bacterial cells is expressed as equivalent amount of cysteine. [b] Least significant difference pairwise comparisons. Values in the same column with different letter superscripts are significantly different ($P < 0.05$).

longum B6, which had the highest reducing activity, equivalent to 225.3 μM of cysteine, is more than 50 times that of *S. thermophilus* 19 987, which had the lowest reducing activity, equivalent to 4.3 μM of cysteine. *L. acidophilus* B, E, N1, and 4356, and *S. thermophilus* 19 987 demonstrated relatively low reducing activity among the 19 strains tested. *L. bulgaricus* and *B. longum* strains tested showed consistently high reducing activity. Generally speaking, most of the strains tested (14 of 19) demonstrated excellent reducing activity.

Superoxide Dismutase (SOD) Activity and SOD Induction. The results of SOD activity of lactic acid bacteria grown in modified MRS containing no added metal ion cofactors and those of induction of SOD of lactic acid bacteria grown in media containing ions are presented in Figure 1. The results were expressed as the inhibition rate of epinephrine autoxidation. Nineteen strains grown in broth containing no added metal ions demonstrated similar inhibition rates in the range 6.2− 8.6%. SOD was not increased when lactic acid bacteria were grown in media containing Mn^{2+}, Fe^{2+}, or $Cu^{2+}Zn^{2+}$ with either method.

DISCUSSION

In the search for antioxidants from natural sources, inhibition of lipid peroxidation is commonly analyzed. Unsaturated fatty acids such as linoleic acid (Wanasundrara et al., 1994), methyl linoleate (Bertelsen et al., 1995), and arachidonic acid (Husain et al., 1987) are typically used. Systems other than lipid peroxidation such as ascorbate autoxidation (Mishra and Korachich, 1984; Rashid et al., 1993) and norepinephrine oxidation (Mishra and Korachich, 1984) are also utilized for analysis. In this study, antioxidative activity was measured based on the inhibition of ascorbate autoxidation. This ascorbate system can be used to not only screen for antioxidants but also test for the presence of adventitious catalytic metals in aqueous phase. The rate of ascorbate oxidation in aqueous solution is highly catalytic metal-dependent (Buettner, 1990). Therefore, modified MRS containing no added metal ion cofactors or deionized water was used for growing cells and preparing intracellular cell-free extract to eliminate the interference of metal ions. Table 1 indicates that the intracellular cell-free extract of all 19 strains possesses antioxidative ability. Further effort was made to identify possible antioxidative mechanisms including metal ion chelating ability, scavenge of reactive oxygen species, reducing activity and superoxide dismutase activity for lactic acid bacteria.

Transition metal ions can initiate lipid peroxidation and start a chain reaction by means such as decomposition of hydroperoxides to give peroxyl and alkoxyl radicals (Halliwell et al., 1995). It has been proposed that iron ion may contribute to the risk of heart disease by promoting free radical production. Cancer and arthritis may also correlate with the catalysis of metal ions (Halliwell and Gutteridge, 1984). Among many metal ions playing catalytic roles, iron and copper ions are highly reactive. Therefore, The chelating ability of lactic acid bacteria toward iron and copper ions was investigated. According to Table 2, *S. thermophilus* 821 demonstrated the best chelating ability for both Fe^{2+} and Cu^{2+} among the 19 strains tested. Other strains also demonstrated chelating ability for either Fe^{2+} or Cu^{2+}. The chelating ability of these strains could be due to the physiological chelators which existed in the intracellular cell-free extract of lactic acid bacteria. Chelators can capture metal ions and prohibit metal ions from catalyzing the oxidation. Many chelators such as EDTA, DETAPAC, BPS, penicillamine, and desferrioxamine have been reported (Gutteridge et al., 1979). Desferrioxamine is an ion chelator isolated from *Strep-*

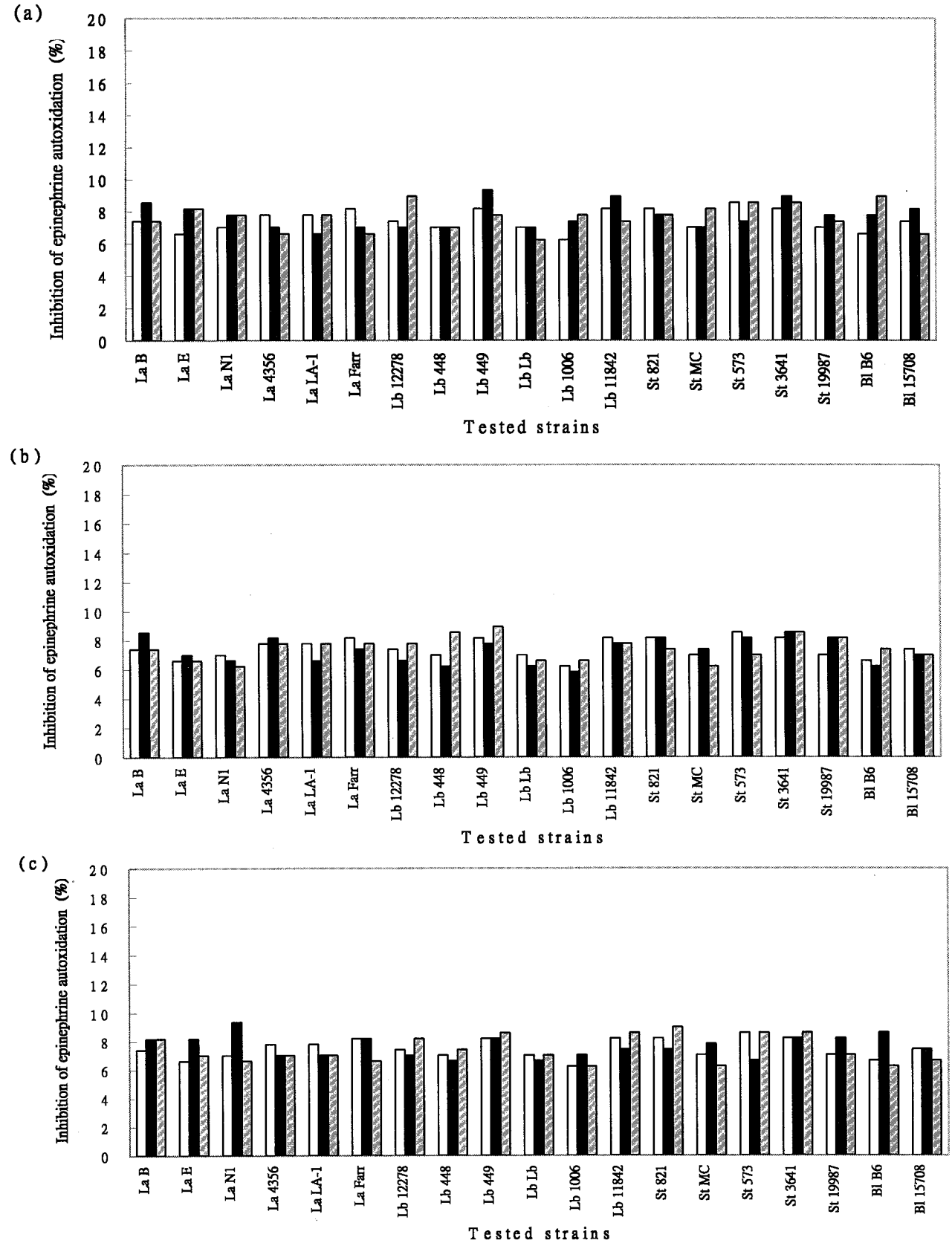

Figure 1. SOD activity of lactic acid bacteria and SOD induction by metal ion Mn^{2+}(a), Fe^{2+}(b), or $Cu^{2+}Zn^{2+}$ (c). Superoxide dismutase activity is expressed as the inhibition rate of epinephrine autoxidation. (White bar) Lactic acid bacteria were grown in modified MRS containing no metal ion cofactors for 18 h. (Black bar) Lactic acid bacteria were grown in modified MRS containing no metal ion cofactors for 18 h and then grown in MRS containing 0.01% Mn^{2+} and shaken for 3 h. (Gray bar) Lactic acid bacteria were grown in modifed MRS plus 0.01% Mn^{2+} for 18 h.

tomyces pilosus. It is available for clinical use in the treatment of thalassemia (Halliwell and Gutteridge, 1989).

To avoid oxidation induced by oxygen radicals, the most efficient means is to scavenge reactive oxygen species. The scavenging ability of lactic acid bacteria for

two of the most important reactive oxygen species, hydroxyl radical and hydrogen peroxide, were investigated. Hydroxyl radical is highly reactive with biological molecules. Hydrogen peroxide is a weak oxidant; however, hydroxyl radical can be generated from hydrogen peroxide when transition metal ions are present (Halliwell, 1994). Organisms capable of producing catalase or peroxidase can degrade hydrogen peroxide. Lim et al. (1993) also reported that the thiol-specific antioxidant protein was found to remove hydrogen peroxide. Data given in Table 4 show that lactic acid bacteria demonstrated different abilities for scavenging hydrogen peroxide. Although lactic acid bacteria are catalase-negative, it has been reported that *B. infantis*, *B. breve*, *B. adolescentis*, and *B. longum* were able to produce NADH-peroxidase to degrade hydrogen peroxide (Shimamura et al., 1992). No enzymes found in organisms are known to degrade hydroxyl radical. Scavengers such as mannitol, formate, and thiourea can protect against damage caused by hydroxyl radical. Uric acid can be used as standard for comparison of scavenging hydroxyl radical ability (Halliwell et al., 1995). As shown in Table 3, lactic acid bacteria demonstrated various degrees of ability for hydroxyl radical scavenge. Since the intracellular cell-free extract is a mixture, it is hard to judge what exactly contributed to the hydroxyl radical scavenge. It would be reasonable to expect that lactic acid bacteria produced scavengers for hydroxyl radical.

Living organisms are also capable of producing antioxidants to reduce the damage of oxidative stress. There are various antioxidants such as NADH, NADPH, glutathione, and uric acid. Proteins and peptides are also antioxidative (Halliwell and Gutteridge, 1989). Ascorbate and α-tocopherol are typical representives of water-soluble and lipid-soluble antioxidant, respectively. Among antioxidants, few are cofactors for antioxidative enzymes, but most inhibit oxidation by reduction. It has been suggested that a substance with reducing activity can decompose hydroperoxides (LOOH) to hydroxyoctadecadienoic acids (LOH) (Gardner, 1975). Table 5 shows that most of the strains of lactic acid bacteria studied possessed high reducing activity. The reducing activity of the intracellular cell-free extract of lactic acid bacteria are probably from the intracellular antioxidants and proteins.

Superoxide dismutase (SOD) is the enzyme responsible for degradation of the toxic superoxides. Although superoxides have limited chemical reactivity, the ability of superoxides to generate more dangerous species, such as highly reactive hydroxyl radicals or the protonated form of superoxides ($HO_2^{\bullet}$) (Halliwell and Chirico, 1993). Decomposition of excess superoxides by SOD is an important physiological antioxidant defense mechanism in aerobic organisms. It has been indicated that *Lactococcus* also expresses antioxidative enzyme SOD activity (Sanders et al., 1995). In this study, all strains demonstrated a rate of inhibition of epinephrine autoxidation in the range 6.2−8.6%; however, since no added metal ions which act as cofactors of SOD enzymes were present, the inhibition of epinephrine autoxidation was likely due to scavenge of superoxides by unknown substances in cells instead of SOD enzymes. Three types of SOD enzymes, classified in terms of their metal cofactors, have been discovered: Mn−SOD, Fe−SOD, and CuZn−SOD (Nakayama, 1992). These metal ion cofactors were thus used to induce SOD enzyme activity in this study. According to the studies of Hansson and Haggstrom (1984) and Smart and Thomas (1987), higher SOD enzyme activity was observed with increased oxygen concentrations in the medium. In the first method for SOD activity induction, lactic acid bacteria were grown in metal ion containing media in a shaker following their growth in modified MRS. The inhibition rate of epinephrine autoxidation was not increased. This indicated that SOD was not induced. Similar results were obtained for lactic acid bacteria grown with the second method, in which lactic acid bacteria were grown in MRS containing metal ions.

The results of this study indicate that lactic acid bacteria have antioxidative ability. Although SOD activity was not found in this study, strains of *L. acidophilus*, *L. bulgaricus*, *S. thermophilus*, and *B. longum* are capable of chelating metal ions, scavenging reactive oxygen species, or possessing reducing activity. Consumption of lactic acid bacteria containing foods may be recommended as healthful. In addition, lactic cultures are potential candidates for production of functional foods or of natural antioxidant supplements.

LITERATURE CITED

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