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Antioxidative Effects of Lactic Acid Bacteria on the Colonic Mucosa of Iron-Overloaded Mice

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The antioxidative effects of lactic acid bacteria on lipid peroxidation in the colonic mucosa were investigated. Among 49 strains of lactic acid bacteria, *Streptococcus thermophilus* YIT 2001 showed the highest inhibitory activity against lipid peroxidation in liposomes induced by ferrous iron. Feeding a diet containing 0.4% *St. thermophilus* YIT 2001 (2×10^8 colony-forming units per mouse per day) for 2 weeks caused a significant decrease of lipid peroxide (thiobarbituric acid reactive substance) in the colonic mucosa of iron-overloaded mice (0.07% Fe in the diet). The mucosal lipid peroxide level did not correlate with the soluble iron concentration of the cecal contents. Therefore, it is suggested that the antioxidative effect of *St. thermophilus* YIT 2001 in the colonic mucosa was not due to the removal of ferrous iron from the reaction system of lipid peroxidation.

KEYWORDS: Colon; lipid peroxide; iron; lactic acid bacteria; antioxidative effect

INTRODUCTION

Several studies have suggested that oxidative damage is an etiological factor in colon cancer and ulcerative colitis (*1*−*5*). Foods containing antioxidative materials may be useful for the prevention of these diseases.

Although iron is an essential nutrient, excessive iron, especially ferrous iron, may cause oxidative damage in tissues. Ferrous iron is a catalyst in the Haber−Weiss reaction, which is hydroxyl radical formation, and is involved in the initiation and propagation of lipid peroxidation. Iron administered orally is not absorbed completely in the upper intestinal tract, and a part (usually >50%) of it reaches the lower intestinal tract. Therefore, oral iron overload causes lipid peroxidation in the colonic mucosa (*6*−*8*).

Lactic acid bacteria, which are facultative anaerobes, have been used to produce dairy products. These bacteria are also becoming popular with consumers due to their benefits to human health. It has been reported that the bacteria or their extracts had antioxidative effects in vitro (*9*−*15*) and a protective effect against hemolysis in vitamin E-deficient rats (*9*). There are, however, few reports on the antioxidative effect of lactic acid bacteria on the colonic mucosa, although the bacteria exist in the intestinal tract and are in contact with the colonic mucosa.

To investigate the antioxidative effect of lactic acid bacteria on the colonic mucosa, we evaluated the inhibitory activity on lipid peroxidation in liposomes among 49 strains of the bacteria and administered two strains to iron-overloaded mice.

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MATERIALS AND METHODS

Bacteria and Culture Conditions. *Enterococcus* (4 strains), *Lactobacillus* (34 strains), *Lactococcus* (2 strains), *Leuconostoc* (2 strains), and *Streptococcus* (7 strains) were obtained from the Culture Collection Research Laboratory of the Yakult Central Institute for Microbiological Research (Tokyo, Japan). The bacteria were cultured in modified GAM broth (Nissui Seiyaku Co., Tokyo, Japan) containing 2% glucose for 18 h at 37 °C. Following saline washes the bacterial cells were harvested by centrifugation at 8500*g* for 15 min. For animal experiments, *Streptococcus thermophilus* YIT 2001 and *Lactobacillus casei* strain Shirota were lyophilized and powdered.

Inhibitory Activity on Lipid Peroxidation in Liposomes. To determine the inhibitory ability of lactic acid bacteria against lipid peroxidation in the cell membrane, we used liposome as the substrate for peroxidation. L-α-Phosphatidylcholine (0.1 g; type XV-E; from egg yolk; Sigma Chemical Co., St. Louis, MO; fatty acid composition: C16:0, 29%; C18:0, 16%; C18:1, 27%; C18:2, 14%; C20:4, 6%) was dissolved in diethyl ether (10 mL), and distilled water (0.6 mL) was added. The mixture was sonicated with an ultrasonic disrupter CA-44882 (Kaijo Co., Tokyo, Japan) while it was evaporated to dryness under a vacuum at 0 °C. Then, 30 mL of 0.1 M *N*-(2-acetamido)-iminodiacetic acid (ADA) sodium buffer (pH 6.7) was added, and the mixture was sonicated for 15 min at 0 °C. The suspension was centrifuged at 1500*g* for 10 min at 4 °C, and the supernatant was used as a liposomal suspension.

The liposomal suspensions were incubated with 0.1 M ADA sodium buffer (pH 6.7), 48 μM ferrous chloride, and 1 mM sodium ascorbate for 2 h at 37 °C in the presence or absence of the bacterial cells. After incubation, lipid peroxide in the mixture was quantified as thiobarbituric acid reactive substances (TBARS) according to the method reported previously (*8*), as follows. In a test tube, 0.1 mL of test sample (liposomal mixture after the incubation), 0.2 mL of 8.1% sodium dodecyl sulfate, 1.5 mL of 20% acetic acid solution adjusted to pH 3.5 with 10 N NaOH, 0.05 mL of 0.8% butylated hydroxytoluene in acetic

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Table 1. Composition (Percent) of Diets[a]

	normal diet group	control group	0.1% group	0.4% group	2.0% group
casein	20.0	18.9	18.9	18.8	18.5
DL-methionine	0.3	0.3	0.3	0.3	0.3
α-corn starch	15.0	15.0	15.0	15.0	14.7
sucrose	50.0	48.9	48.8	48.6	47.6
cellulose fiber	5.0	5.0	5.0	5.0	4.9
corn oil	5.0	5.0	5.0	5.0	4.9
AIN-76 mineral mix	3.5	3.5	3.5	3.5	3.4
AIN-76 vitamin mix	1.0	1.0	1.0	1.0	1.0
choline bitartrate	0.2	0.2	0.2	0.2	0.2
lactic acid bacteria	0	0	0.1	0.4	2.0
skim milk	0	2.0	2.0	2.0	2.0
ferrous fumarate	0	0.2	0.2	0.2	0.2

[a] Iron concentration in diets was 0.07% except in the normal diet (0.0035%).

acid, 1.0 mL of 0.8% thiobarbituric acid, 0.7 mL of 5 mM ethylenediaminetetraacetic acid (EDTA), and 0.5 mL of distilled water were added, in that order. This mixture was kept at 5 °C for 60 min and then heated to 100 °C for 60 min. TBARS were extracted with 5.0 mL of *n*-butanol−pyridine (15:1 v/v) and quantified from the fluorescence intensity (excitation, 515 nm; emission, 553 nm; RF-5300PC spectrofluorophotometer, Shimadzu Co., Kyoto, Japan).

The inhibition rate for lipid peroxidation was calculated according to the equation

$$\text{inhibition rate for lipid peroxidation} = (C - T)/(C - B) \times 100$$

where C = TBARS of a test sample incubated without the bacterial cells, T = TBARS of a test sample incubated with the bacterial cells, and B = TBARS of distilled water.

The inhibition rate was examined at four or five bacterial concentrations. Bacterial concentration was expressed as viable bacterial number (colony-forming units, cfu) per milliliter, which was calculated from the colony count on modified GAM agar plates (Nissui Pharmaceutical Co., Tokyo, Japan) after incubation at 37 °C for 2 days. The bacterial concentration was converted to the common logarithm of cfu/mL. The bacterial concentration causing 50% inhibition of lipid peroxidation (50% inhibitory concentration, IC_{50}) was calculated from the sigmoidal inhibition curve.

Animals and Diets. Two animal experiments were performed that were identical except for the bacterial strain contained in the diet. Animal experiments 1 and 2 used *St. thermophilus* YIT 2001 and *Lb. casei* strain Shirota, respectively.

Six-week-old male BALB/cA mice were obtained from Clea Japan, Inc., Tokyo, Japan. After acclimation for 1 week, the mice were randomly divided into five groups, each containing 12 mice, and fed the following diets: AIN-76 (*16*) diet for the normal diet group; iron-enriched (control) diet for the control group; iron-enriched diet containing 0.1% lyophilized bacteria for the 0.1% group; iron-enriched diet containing 0.4% lyophilized bacteria for the 0.4% group; and iron-enriched diet containing 2.0% lyophilized bacteria for the 2.0% group (**Table 1**). The dietary treatment was continued for 2 weeks.

The mice had free access to food and water and were accommodated in a room with controlled temperature (25 °C), humidity (55%), and light (12-h light/dark cycle) during the whole experimental period. Body weight was recorded every week.

After the dietary treatment, the mice were sacrificed. They were anesthetized with Nembutal (Abbott Laboratories, North Chicago, IL), and blood was obtained from the inferior vena cava and mixed with heparin. The colon and cecum were removed with their contents.

All animal procedures were approved by the Ethical Committee for Animal Experiments of Yakult Central Institute.

Preparation of Plasma and Colonic Mucosa, and Treatments of Cecal Contents. Plasma was obtained from heparinized blood by centrifugation at 1500*g* for 15 min. The colon was incised and rinsed with saline. The colonic mucosa was scraped off with a clean glass microscope slide and homogenized in 1.15% KCl. The cecum was incised, and the contents were recovered. The cecal contents were dispersed in saline (sample: saline = 1:10 v/v) and centrifuged at 13000*g* for 15 min. The supernatant was recovered to measure soluble iron (total and ferrous iron) concentrations.

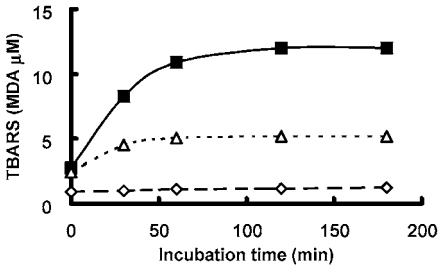

Figure 1. Time course of lipid peroxidation (levels of TBARS) in liposomes: (■) liposomes incubated at 37 °C with 48 μM ferrous chloride; (◇) liposomes incubated at 37 °C without ferrous chloride; (△) liposomes incubated at 37 °C with 48 μM ferrous chloride and *St. thermophilus* YIT 2001 (3×10^8 cfu/mL).

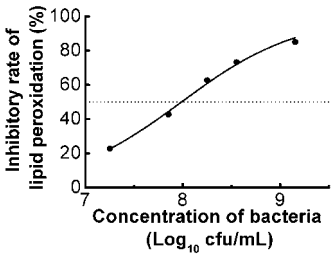

Figure 2. Relationship between inhibition rate of lipid peroxidation in liposomes and concentration of *St. thermophilus* YIT 2001. The liposomes were incubated for 2 h at 37 °C. The inhibition curve was calculated from sigmoidal curve fitting.

Biochemical Analysis. Mucosal lipid peroxide was measured using the TBARS assay reported previously (*8*), in the same way as for the in vitro assay. Protein concentration was measured with a BCA protein assay kit (Pierce Co., Rockford, IL).

Iron concentrations in plasma and cecum contents were measured by a colorimetric method using a commercial kit (Fe-C test Wako, Wako Pure Chemical Industries, Tokyo, Japan). Ferrous iron concentration was measured with the Fe-C test Wako without using a reductant.

Statistics. Data were reported as mean ± standard deviation (SD). Dunnet's multiple-comparison test was used for statistical analysis of inhibitory activity of the bacteria on lipid peroxidation in liposomes. Data obtained from the animal experiments were analyzed using one-way analysis of variance (ANOVA). When the result of ANOVA was significant ($P < 0.05$), Tukey's multiple-comparison tests were conducted. The SAS system for Windows (release 6.12; SAS Institute Inc., Cary, NC) was employed as software for the statistical analysis.

RESULTS

Inhibitory Activity on Lipid Peroxidation in Liposomes. The inhibitory activity of the lactic acid bacteria on lipid peroxidation in liposomes was examined. The quantity of lipid peroxide (TBARS) produced by this reaction system was almost leveling off after an incubation of 120 min (**Figure 1**). The TBARS decreased depending on the bacterial concentration in every strain we tested. As a typical example, **Figure 2** shows the relationship between inhibitory rate of lipid peroxidation and bacterial concentration of *St. thermophilus* YIT 2001. The IC_{50} of the tested strains ranged from 7.9×10^7 cfu/mL ($\log_{10}$ 7.9) to 1.0×10^{10} cfu/mL ($\log_{10}$ 10.0). Among the 49 strains, *St. thermophilus* YIT 2001 most effectively inhibited lipid peroxidation (**Table 2**).

Suppressive Effects on Lipid Peroxidation in Colonic Mucosa. Figure 3 shows the effect of administration of *St. thermophilus* YIT 2001 as a dietary admixture on lipid peroxi-

Table 2. Fifty Percent Inhibitory Concentrations (IC_{50}) of Lactic Acid Bacteria for Lipid Peroxidation in Liposomes

strain[a]	IC_{50}[b] (log_{10} cfu/mL)
St. thermophilus YIT 2001	7.9 ± 0.2
Lb. acidophilus YIT 0070	8.1 ± 0.1
Lb. delbrueckii ssp. *bulgaricus* YIT 0447	8.2 ± 0.4
Lb. delbrueckii ssp. *bulgaricus* YIT 0098	8.2 ± 0.2
Lb. delbrueckii ssp. *bulgaricus* YIT 0206	8.3 ± 0.4
Lb. bifermentans YIT 0260	8.3 ± 0.3
Lb. salivarius ssp. *salicinium* YIT 0089	8.4 ± 0.2
Lb. delbrueckii ssp. *bulgaricus* YIT 0162	8.4 ± 0.2
Lb. paraplantarum YIT 0445	8.4 ± 0.3
St. thermophilus YIT 2042	8.4 ± 0.2
Lb. salivarius ssp. *salivarius* YIT 0104	8.4 ± 0.3
Lb. delbrueckii ssp. *bulgaricus* YIT 0181	8.4 ± 0.3
Lb. delbrueckii ssp. *bulgaricus* YIT 0182	8.5 ± 0.3
St. thermophilus YIT 2045	8.6 ± 0.3 *
Lb. casei strain Shirota	8.7 ± 0.2 *
Lb. delbrueckii ss. *bulgaricus* YIT 0044	8.7 ± 0.2 *
Lb. crispatus YIT 0212	8.9 ± 0.3 **
Lb. helveticus YIT 0083	8.9 ± 0.2 **
Ec. faecalis YIT 2031	8.9 ± 0.1 **
Lb. fermentum YIT 0453	9.0 ± 0.2 **
Leuc. lactis YIT 3001	9.0 ± 0.2 **
Lb. delbrueckii ssp. *bulgaricus* YIT 0067	9.0 ± 0.3 **
Ec. faecalis YIT 2113	9.0 ± 0.3 **
St. thermophilus YIT 2046	9.0 ± 0.2 **
Lb. gasseri YIT 0192	9.0 ± 0.2 **
Lc. lactis ssp. *lactis* YIT 2008	9.0 ± 0.4 **
Lc. lactis YIT 2027	9.1 ± 0.3 **
Lb. gasseri YIT 0168	9.1 ± 0.3 **
Leuc. pseudomesenteroides YIT 2077	9.1 ± 0.1 **
St. thermophilus YIT 2021	9.1 ± 0.2 **
Ec. faecium YIT 2032	9.1 ± 0.5 **
Lb. plantarum YIT 0102	9.2 ± 0.1 **
Lb. zeae YIT 0078	9.3 ± 0.4 **
St. thermophilus YIT 2086	9.3 ± 0.5 **
Lb. vaginalis YIT 0276	9.3 ± 0.4 **
Lb. reuteri YIT 0197	9.3 ± 0.3 **
Lb. rhamnosus YIT 0105	9.4 ± 0.2 **
Lb. sake YIT 0247	9.4 ± 0.3 **
Lb. fermentum YIT 0081	9.4 ± 0.2 **
Lb. buchneri YIT 0077	9.5 ± 0.4 **
Lb. salivarius YIT 0452	9.6 ± 0.2 **
Lb. casei YIT 0180	9.6 ± 0.3 **
St. thermophilus YIT 2084	9.6 ± 0.1 **
Lb. brevis YIT 0076	9.7 ± 0.1 **
Lb. aviarius ssp. *araffinosus* YIT 0257	9.8 ± 0.3 **
Lb. suebicus YIT 0275	9.8 ± 0.3 **
Lb. casei ss. *paracasei* YIT 0209	9.8 ± 0.3 **
Lb. curvatus YIT 0234	9.8 ± 0.1 **
Ec. faecium YIT 2112	10.0 ± 0.2 **

[a] *St.*, *Streptococcus*; *Lb.*, *Lactobacillus*; *Lc.*, *Lactococcus*; *Ec.*, *Enterococcus*; *Leuc.*, *Leuconostoc*. [b] IC_{50} values are expressed as the logarithm of bacterial count per milliliter. Values are mean ± SD ($n = 3$). *, $P < 0.05$; **, $P < 0.01$ (Dunnet's multiple-comparison test; compared with *St. thermophilus* YIT 2001).

dation in the colonic mucosa of iron-overloaded mice. The mucosal TBARS of iron-overloaded mice was increased as compared to that of mice fed a normal diet ($P < 0.05$). TBARS of iron-overloaded mice fed a diet containing *St. thermophilus* YIT 2001 (0.1−2.0%) was decreased depending on the bacterial concentration in the diet, and a significant difference was observed between the control group and the S 0.4% group or S 2.0% group. Mice in the S 0.4% group were fed the bacteria at 2×10^8 cfu/mouse/day. *Lb. casei* strain Shirota significantly decreased TBARS in the colonic mucosa of iron-overloaded mice at a bacterial concentration of 2.0% in the diet and caused no significant decrease at 0.4% (3×10^8 cfu/mouse/day) (**Figure 4**).

Iron Concentration in Cecal Contents and Plasma. There was no significant difference between the control group and each bacteria-fed group (S 0.1%, S 0.4%, S 2.0%) in iron concentration in the cecal contents and plasma in animal experiment 1 (**Table 3**). In animal experiment 2, the feeding of *Lb. casei* strain Shirota did not affect total iron concentration in the cecal contents (**Table 4**). Although ferrous iron concentrations in the cecal contents of the Lb 0.1% group and Lb 0.4% group were increased and the plasma iron concentration of the Lb 0.4% group was decreased compared with those of the control group, these changes did not depend on the bacterial concentration in the diet.

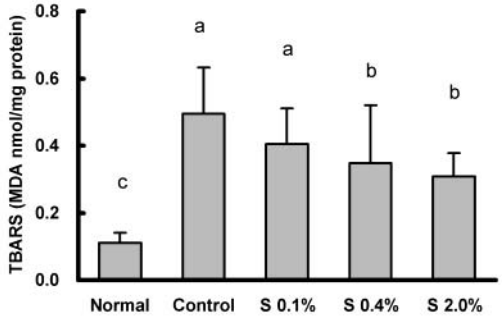

Figure 3. Effects of *St. thermophilus* YIT 2001 on levels of lipid peroxide (TBARS) in the colonic mucosa of mice (animal experiment 1). Mice were fed an AIN-76 diet (normal), iron-enriched diet (control), 0.1% *St. thermophilus* YIT 2001-containing iron-enriched diet (S 0.1%), 0.4% *St. thermophilus* YIT 2001-containing iron-enriched diet (S 0.4%), or 2.0% *St. thermophilus* YIT 2001-containing iron-enriched diet (S 2.0%). Different letters indicate a significant difference ($P < 0.05$).

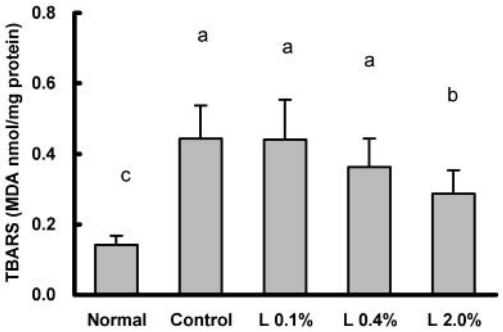

Figure 4. Effects of *Lb. casei* strain Shirota on levels of lipid peroxide (TBARS) in colonic mucosa of mice (animal experiment 2). Mice were fed an AIN-76 diet (normal), iron-enriched diet (control), 0.1% *Lb. casei* strain Shirota-containing iron-enriched diet (L 0.1%), 0.4% *Lb. casei* strain Shirota-containing iron-enriched diet (L 0.4%), or 2.0% *Lb. casei* strain Shirota-containing iron-enriched diet (L 2.0%). Different letters indicate a significant difference ($P < 0.05$).

There were no significant correlations between soluble iron (total or ferrous) concentrations in the cecum contents and mucosal TBARS of iron-overloaded mice in both animal experiments. Correlation coefficients (r) between the total iron and the TBARS were 0.131 and −0.151 in animal experiments 1 and 2, respectively. Correlation coefficients (r) between the ferrous iron and the TBARS were −0.091 and −0.059 in animal experiments 1 and 2, respectively.

Body Weight of Mice. No significant difference was observed in body weight among the five groups in both animal experiments.

DISCUSSION

The antioxidative activity of lactic acid bacteria was evaluated by determining their inhibitory activity on lipid peroxidation

Table 3. Effects of *Streptococcus thermophilus* YIT 2001 on Iron Concentration in Cecal Contents and Plasma (Animal Experiment 1)[a]

	normal	control	S 0.1%	S 0.4%	S 2.0%
soluble iron concn in cecal contents (mg/dL)					
total iron	0.53 ± 0.12b	5.19 ± 3.10a	3.61 ± 3.92ab	4.43 ± 1.38a	3.05 ± 1.71ab
ferrous iron	0.19 ± 0.05c	0.91 ± 0.35ab	0.70 ± 0.50b	1.14 ± 0.31a	0.84 ± 0.35ab
iron concn in plasma (mg/dL)					
total iron	0.30 ± 0.04a	0.30 ± 0. 12a	0.35 ± 0.08a	0.37 ± 0.04a	0.33 ± 0.12a

[a] Values are mean ± SD for 12 mice. Mean values within a row not sharing a common letter are significantly different ($P < 0.05$). Mice were fed an AIN-76 diet (normal), iron-enriched diet (control), 0.1% *St. thermophilus* YIT 2001-containing iron-enriched diet (S 0.1%), 0.4% *St. thermophilus* YIT 2001-containing iron-enriched diet (S 0.4%), or 2.0% *St. thermophilus* YIT 2001-containing iron-enriched diet (S 2.0%).

Table 4. Effects of *Lactobacillus casei* Strain Shirota on Iron Concentrations in Cecal Contents and Plasma (Animal Experiment 2)

	normal	control	Lb 0.1%	Lb 0.4%	Lb 2.0%
soluble iron concn in the cecal contents (mg/dL)					
total iron	0.80 ± 0.24b	2.81 ± 0.97ab	4.43 ± 2.01a	2.79 ± 1.73ab	4.20 ± 2.35a
ferrous iron	0.31 ± 0.06d	0.76 ± 0.18c	1.15 ± 0.44ab	0.85 ± 0.33bc	1.29 ± 0.42a
iron concn in plasma (mg/dL)					
total iron	0.45 ± 0.04bc	0.50 ± 0.03ac	0.50 ± 0.02ac	0.45 ± 0.03b	0.52 ± 0.05a

[a] Values are mean ± SD for 12 mice. Mean values within a row not sharing a common letter are significantly different ($P < 0.05$). Mice were fed an AIN-76 diet (normal), iron-enriched diet (control), 0.1% *Lb. casei* strain Shirota-containing iron-enriched diet (Lb 0.1%), 0.4% *Lb. casei* strain Shirota-containing iron-enriched diet (Lb 0.4%), or 2.0% *Lb. casei* strain Shirota-containing iron-enriched diet (Lb 2.0%).

induced by ferrous iron in liposomes. Among 49 strains of lactic acid bacteria, *St. thermophilus* YIT 2001 most effectively inhibited lipid peroxidation. Therefore, we investigated the effect of *St. thermophilus* YIT 2001 on the colonic mucosa of iron-overloaded mice. In additon to this strain, we chose *Lb. casei* strain Shirota for in vivo experiments. *Lb. casei* strain Shirota is a potent probiotic bacteria reported to have many beneficial functions, such as control of gastrointestinal infections (*17*, *18*), stimulation of the immune system (*19*, *20*) and antitumor activity (*21*, *22*). Oral administration of these strains decreased TBARS in the colonic mucosa of iron-overloaded mice at different bacterial doses. *St. thermophilus* YIT 2001 significantly decreased TBARS in the colonic mucosa at a bacterial concentration of 0.4% in the diet (**Figure 1**), whereas *Lb. casei* strain Shirota required 2.0% for a significant decrease of TBARS (**Figure 2**). This difference of in vivo antioxidative activity between the two strains was consistent with their in vitro antioxidative activities, which for *St. thermophilus* YIT 2001 was 6 times ($\log_{10}$ 0.8) higher than for *Lb. casei* strain Shirota.

Some researchers (*9*−*15*) have reported in vitro antioxidative activity of lactic acid bacteria. Kaizu et al. (*9*) reported that hemolysis of erythrocytes was inhibited in rats administered an intracellular extract of lactobacilli. The authors of these reports used a cell-free extract as the material and considered that the extract was absorbed from the small intestine and acted in the blood and tissues. However, it is hypothesized that non-absorbable antioxidants are effective against colonic oxidative stress (*3*, *23*). Lactic acid bacteria may act as a non-absorbable antioxidant in the colon or at the surface of colonic mucosa, unlike their extracts.

We have previously reported the antioxidative activity of bifidobacteria, which are obligate anaerobes (*8*). The lactic acid bacteria we tested are facultative anaerobes that can grow in oxygen-containing environments. Therefore, we expected that they would have higher antioxidative activity than bifidobacteria. Although the highest inhibitory activity of liposome peroxidation was obtained with one of the lactic acid bacteria, *St. thermophilus* YIT 2001, the range of IC_{50} of lactic acid bacteria (from 7.9×10^7 to 1.0×10^{10} cfu/mL; **Table 2**) overlapped that of bifidobacteria (from 2.0×10^8 to 4.0×10^9 cfu/mL; cited from the ref *8*). It was suggested that the antioxidative activity did not correlate directly with the ability of growth under aerobic conditions for bifidobacteria and lactic acid bacteria.

Lipid peroxidation was accelerated by the catalytic activity of ferrous iron. Dietary phytate, which chelates ferrous iron to generate a form that does not support the Haber−Weiss reaction, inhibited lipid peroxidation in the colonic mucosa (*7*). Kot et al. (*24*) reported that intestinal bacteria had iron-incorporating (accumulating) activity. Therefore, the antioxidative mechanism may include iron chelation to generate a form that does not support the Haber−Weiss reaction or incorporation of iron into bacterial cells. However, the mucosal lipid peroxide level did not correlate with soluble iron concentration in the cecal contents. It was suggested that the antioxidative effect of *St. thermophilus* YIT 2001 in the colonic mucosa was not based on the action of ferrous iron removal (chelation, incorporation in bacterial cells, or oxidation) from the reaction system of lipid peroxidation. Therefore, *St. thermophilus* YIT 2001 may protect the colonic mucosa from oxidative damage without inhibiting iron absorption, whereas phytate is a potent inhibitor of iron absorption due to its chelating activity (*25*, *26*). *St. thermophilus* YIT 2001 might act as scavenger of reactive oxygen or free radicals and increase antioxidative capacities of intestinal contents. To explain the mechanism of the antioxidative effect, further investigations such as determination of active component are needed.

Sawa et al. (*27*) reported oxidative stress caused by simultaneous feeding of a fat diet and that heme-iron increased the incidence of colon cancer in rats given *N*-nitroso-*N*-methylurea. Siegers et al. (*28*) and Nelson et al. (*29*) reported dietary iron (ferrous fumarate or ferrous sulfate) enhanced the incidence of colon cancer induced by dimethylhydrazine in a mouse model. We showed that *St. thermophilus* YIT 2001 protected the colonic mucosa against oxidative stress. Oxidative stress in the colonic mucosa is presumably involved in the pathogenesis of ulcerative colitis and colon cancer (*1*−*5*). *St. thermophilus* YIT 2001 may decrease the incidence of these diseases.

ABBREVIATIONS USED

ADA, *N*-(2-acetamido)iminodiacetic acid; ANOVA, analysis of variance; cfu, colony-forming units; EDTA, ethylenediamine-

tetraacetic acid; IC_{50}, 50% inhibitory concentration; TBARS, thiobarbituric acid reactive substance.

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