Preventive Effect of *Streptococcus thermophilus* YIT 2001 on Dextran Sulfate Sodium-Induced Colitis in Mice

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We investigated the preventive effect of *Streptococcus thermophilus* YIT 2001, a lactic acid bacterum having high antioxidative activity, on acute colitis induced by 2.5% dextran sulfate sodium in mice, and compared the effect with that of *S. thermophilus* YIT 2084 which has lower antioxidative activity. Feeding *S. thermophilus* YIT 2001 decreased the disease activity index and level of lipid peroxide (the thiobarbituric acid reactive substance content) in the colonic mucosa. The hematocrit and hemoglobin concentrations in the blood of *S. thermophilus* YIT 2001-fed mice were higher than those of the control mice. *S. thermophilus* YIT 2084 had no significant effect on these parameters. The results suggest that the antioxidative activity of *S. thermophilus* YIT 2001 was involved in the improving effect on colitis.

**Key words:** lactic acid bacteria; oxidative injury; dextran sulfate sodium; ulcerative colitis; antioxidative activity

Ulcerative colitis is characterized by the infiltration of neutrophils, monocytes and lymphocytes into the colon and rectum, its main symptoms being diarrhea, bloody stools, abdominal pain, weight loss and anemia. Recent studies have suggested that the enhanced release of reactive oxygen species played an important role in the pathogenesis of ulcerative colitis. The inflamed colonic mucosa from ulcerative colitis patients is rich in activated neutrophils, monocytes and lymphocytes, and these cells generate excessive amounts of reactive oxygen species. The increased generation of highly reactive oxygen species exceeds the capacity of the limited intestinal antioxidative defense system, thereby contributing to intestinal oxidative injury in ulcerative patients. The administration of dextran sulfate sodium (DSS) via drinking water to rats or mice has induced colitis lesions that resembled those of human ulcerative colitis in both symptomatic and histological findings, and the beneficial effects of some antioxidants and free radical scavengers on DSS-induced colitis have been reported. Lactic acid bacteria are used to produce dairy products. We have previously reported that *Streptococcus thermophilus* YIT 2001 showed the highest in vitro antioxidative activity against lipid peroxidation in a cell membrane model among 49 strains of lactic acid bacteria, and feeding this strain decreased the oxidative injury to the colonic mucosa of iron-overloaded mice. Meanwhile, the in vitro antioxidative activity of *S. thermophilus* YIT 2084 was one-fiftieth that of *S. thermophilus* YIT 2001. Recent studies have demonstrated that lactic acid bacteria having anti-oxidative activity, such as recombinants producing superoxide dismutase, reduced the severity of experimental colitis.

In the present study, we investigated the preventive effect of *S. thermophilus* YIT 2001 on DSS-induced acute colitis in mice, and compared it with the effect of *S. thermophilus* YIT 2084 which has lower antioxidative activity.

**Materials and Methods**

Bacteria and culture conditions. *Streptococcus thermophilus* YIT 2001 and YIT 2084 were obtained from the Culture Collection Research Laboratory of Yakult Central Institute for Microbiological Research (Tokyo, Japan). The bacteria were cultured for 18 h at 37 °C in modified GAM broth (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) containing 2% lactose. Following saline washing, the bacterial cells were harvested by centrifugation at 8,500 g for 15 min and then lyophilized.

Animals and diets. All the animal experiments were performed in accordance with the guidelines of the Ethical Committee for Animal Experiments of Yakult Central Institute for Microbiological Research (Tokyo, Japan).

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**Abbreviations:** DSS, dextran sulfate sodium; SAA, serum amyloid A; TBARS, thiobarbituric acid reactive substance; MDA, malondialdehyde; MPO, myeloperoxidase
Seven-week-old male BALB/cA mice were obtained from Clea Japan, Inc., Tokyo, Japan. The mice had free access to food and water, and were accommodated in a room with controlled temperature (25°C) and humidity (55%) and light (12 h light/dark cycle) during the whole experimental period. After acclimatization for 1 week, the mice were randomly assigned (n = 11-12) to four (Experiment 1) or three (Experiment 2) groups (at day 1). The AIN-76 diet,11) with added 2% skimmed milk as a protectant for lyophilizing the bacterial cells, was used for the basal diets (Table 1). The mice were fed on the experimental diets from day 1 to day 14.

To induce acute colitis, the mice were given a 2.5% dextran sulfate sodium (DSS; molecular weight 36000–50000, ICN Biomedicals, Inc., Aurora, OH, USA) solution as drinking water ad libitum from day 8 to day 14.

The mice were sacrificed on day 14. They were anesthetized with Nembutal (Abbott Laboratories, North Chicago, IL, USA), and blood was obtained from the inferior vena cava. The colon was removed, incised, and rinsed with saline.

**Disease activity index.** The disease activity index was estimated by scoring the changes in weight, hemoccult positivity or gross bleeding and stool consistency according to the method of Cooper et al.12) Occult blood in the feces was measured with a commercial kit (Bensenketsu slide 5 Shionogi 2, Shionogi & Co., Ltd., Osaka, Japan).

**Biochemical analysis.** The hemoglobin concentration was measured with a Hemoglobin B-Test Wako kit (Wako Pure Chemical Industries Ltd., Tokyo, Japan). Hematocrit was measured by centrifuging blood collected into heparinized microcapillary tubes. Serum amyloid A (SAA) was measured with a commercial ELISA kit (Mouse SAA immunoassay kit, BioSource International, Inc., Camarillo, CA, USA).

Colonic mucosa was scraped off with a clean glass microscope slide and homogenized in 1.15% KCl. Mucosal lipid peroxide was measured by using the thioarbituric acid reactive substance (TBARS) assay reported previously.13) TBARS is expressed as the malondialdehyde (MDA) equivalent. The mucosal myeloperoxidase (MPO) activity was measured by the method of Krawisz et al.14) One unit of MPO activity is defined as that degrading 1 µmol of peroxide per minute at 25°C.

The protein concentration was measured with a BCA protein assay kit (Pierce Chemical Co., Rockford, IL, USA).

**Statistics.** Statistical analyses were performed by using the SAS system for Windows (release 6.12; SAS Institute, Inc., Cary, NC, USA). The data, except for the disease activity index, were subjected to a one-way analysis of variance (1-way ANOVA) and Dunnett’s multiple comparison test. Differences in the disease activity index between groups were evaluated by a Kruskal–Wallis test and non-parametric Dunnett’s multiple comparison test. Each data value is expressed as the mean ± standard deviation.

### Results

**The relationship between the concentration of S. thermophilus YIT 2001 in the diet and the protective effect on colitis (Experiment 1)**

Decreases in the disease activity index were observed in the mice fed with the diets containing S. thermophilus YIT 2001 in a dose-dependent manner (Table 2). The oxidative injury to the colonic mucosa was evaluated by measuring the thioarbituric acid reactive substance (TBARS) formation. TBARS values in the mice with induced colitis were 1.5-fold higher compared than those that in the non-induced mice (MDA 0.12 ±
Discussion

Reactive oxygen species have been implicated as a factor contributing to tissue destruction in ulcerative colitis. Some studies have reported that oxidative injury occurred in the colonic mucosa of patients or animal models of ulcerative colitis.\(^1\sim5\) We have reported previously that \textit{Streptococcus thermophilus} YIT 2001, a strain of lactic acid bacteria used to produce fermented milk, protected the colonic mucosa from oxidative injury in iron-overloaded mice.\(^8\) In this present study, \textit{S. thermophilus} YIT 2001 decreased the content of lipid peroxide in colonic mucosa and improved the symptoms of DSS-induced mouse colitis. Since \textit{S. thermophilus} YIT 2084, which had lower antioxidative activity (one-fiftieth of that of \textit{S. thermophilus} YIT 2001),\(^5\) showed no effect on the colitis, it would appear that the antioxidative activity of \textit{S. thermophilus} YIT 2001 was involved in the colitis improvement.

Recent studies have demonstrated that lactic acid bacteria producing superoxide dismutase reduce the severity of experimental colitis.\(^9\sim10\) However, it might be low possibilities that an enzyme such as superoxide dismutase was involved in the antioxidative activity of \textit{S. thermophilus} YIT 2001, because heat treatment (100°C for 15 min) of this strain under anaerobic conditions did not change the \textit{in vitro} antioxidative activity against the lipid peroxidation in liposomes (data not shown).

Inflamed colonic mucosa from ulcerative colitis patients and murine models are rich in activated neutrophils containing myeloperoxidase (MPO). Serum amyloid A (SAA) is an acute-phase protein primarily produced by the liver in response to IL-1, IL-6 and tumor necrosis factor. \textit{S. thermophilus} YIT 2001 had no significant effect on MPO activity in the colonic mucosa nor on SAA concentration. Iwai and Iwashita\(^15\) have reported that rebamipide, which inhibits the production of free radicals, protected against DSS-induced mucosal damage in rats, but did not protect against the infiltration of neutrophils in rat rectal mucosa exposed to DSS. Gonzalez \textit{et al.}\(^16\) have reported that dietary vitamin E supplementation improved the diarrhea that accompanied colitis induced by 2,4-trinitrobenzenesulfonic acid in rats, but that it brought about no change in MPO activity. Some antioxidants, similar to \textit{S. thermophilus} YIT 2001, might improve colitis by acting directly on the scavengers of reactive oxygen species produced by neutrophils, rather than by inhibiting neutrophil infiltra-

\begin{table}[h]
\centering
\caption{Relationship between the Concentration of \textit{S. thermophilus} YIT 2001 in the Diet and the Protective Effect on Colitis (Experiment 1)\(^*\)}
\footnotesize
\begin{tabular}{|c|c|c|c|}
\hline
Parameter & Control group & 0.1% group & 0.4% group & 2.0% group \\
\hline
Disease activity index & 9.1 ± 0.7 & 9.0 ± 0.8 & 7.5 ± 2.0 & 6.8 ± 2.5* \\
Level of lipid peroxide & 0.20 ± 0.08 & 0.17 ± 0.03 & 0.14 ± 0.02* & 0.14 ± 0.04* \\
(TBARS) in colonic mucosa & & & & \\
(MDA nmol/mg of protein) & & & & \\
Colon length (mm) & 67.7 ± 9.2 & 65.9 ± 7.1 & 69.1 ± 9.1 & 62.6 ± 10.2 \\
Weight gain for period of DSS & −2.9 ± 3.1 & −3.2 ± 3.5 & −2.2 ± 3.3 & −3.1 ± 3.4 \\
administration (day 8 to day 14) (g) & & & & \\
\hline
\end{tabular}
\footnotesize{\(^* P < 0.05: \text{Dunnett's multiple-comparison test}\)}
The disease activity index was estimated by scoring changes in the weight, hemoccult positivity or gross bleeding and stool consistency according to the method of Cooper \textit{et al.}\(^10\).}

\end{table}

0.01 nmol/mg of protein). TBARS values in the colitis mice fed on a diet containing \textit{S. thermophilus} YIT 2001 (0.1%–2.0%) were lower depending on the microorganism concentration in the diet. Significant differences in mucosal TBARS values were observed between the control group and the 0.4% and 2.0% groups. The mice in the 0.4% group were fed with the bacteria at 2 \times 10^8 \text{cfu/mouse/day}. The disease activity index was positively correlated with the TBARS value in the colonic mucosa (R = 0.43, P < 0.01).

Comparison of the protective effects on colitis between strains with different antioxidative activities (Experiment 2)

Figure 1A shows the disease activity index of the colitis mice fed with a diet containing \textit{S. thermophilus} YIT 2001 or YIT 2084. The disease activity index of the YIT 2001 group is significantly lower than that of the control group, although there is no difference between the control group and YIT 2084 group.

TBARS values in the colonic mucosa of the YIT 2001 group are significantly lower. However, YIT 2084 had no significant effect on TBARS in the colonic mucosa of the colitis mice (Fig. 1B). Neither \textit{S. thermophilus} YIT 2001 nor YIT 2084 had any significant effect on myeloperoxidase activity in the colonic mucosa or on the serum amyloid A concentration (Table 3).

The effect of antioxidative bacteria on the anemia resulting from colitis was also evaluated. The hemoglobin concentration and hematocrit (Fig. 2) in blood of the YIT 2001 group were higher than those in the control group. \textit{S. thermophilus} YIT 2084 had no significant effect on anemia in terms of either parameter.
tion or inflammatory cytokine production, although it has been reported that other antioxidants decreased the MPO activity in acute colitis models.1,7) *S. thermophilus* YIT 2001 reduced the anemia resulting from DSS-induced colitis. Although the mucosal bleeding associated with active colitis often causes iron-deficiency anemia, it has been reported that iron supplementation enhanced the oxidative stress and disease activity with induced colitis in rats.17) We have previously reported that *S. thermophilus* YIT 2001 protected the colonic mucosa from oxidative injury catalyzed by iron.8) Combining this strain with iron supplementation might provide a means for not only preventing but also for treating the anemia associated with colitis.

In conclusion, *S. thermophilus* YIT 2001, which has high antioxidative activity, prevented oxidative injury to the colonic mucosa and improved the disease activity index in DSS-induced colitis mice. Furthermore, anemia following this colitis was reduced by the ingestion of this lactic acid bacterium. *S. thermophilus*

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**Table 3. Mucosal MPO Activity, SAA, Colon Length and Weight Gain of Colitis Mice (Experiment 2)**

<table>
<thead>
<tr>
<th></th>
<th>Control group</th>
<th>YIT 2001 group</th>
<th>YIT 2084 group</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO activity (unit/g of protein)</td>
<td>25.7 ± 17.2</td>
<td>19.3 ± 9.3</td>
<td>30.7 ± 31.3</td>
</tr>
<tr>
<td>Serum amyloid A (mg/ml)</td>
<td>4.5 ± 5.4</td>
<td>2.8 ± 4.1</td>
<td>6.4 ± 6.4</td>
</tr>
<tr>
<td>Colon length (mm)</td>
<td>63.5 ± 7.8</td>
<td>72.6 ± 8.8*</td>
<td>65.2 ± 7.8</td>
</tr>
<tr>
<td>Weight gain for period of DSS administration (day 8 to day 14) (g)</td>
<td>−1.7 ± 1.6</td>
<td>−0.7 ± 1.9</td>
<td>−1.6 ± 1.6</td>
</tr>
</tbody>
</table>

*P < 0.05: Dunnett’s multiple-comparison test

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**Fig. 1.** Disease Activity Index and Colonic Mucosal TBARS Value of Colitis Mice Fed on a Diet Containing *S. thermophilus* YIT 2001 or YIT 2084 (Experiment 2).

Mice were fed on a control diet (Control), a diet containing 0.4% *S. thermophilus* YIT 2001 (YIT 2001), or a diet containing 0.4% *S. thermophilus* YIT 2084 (YIT 2084). A, Disease activity index of DSS-induced colitis mice. The disease activity index was estimated by scoring changes in the weight, hemoccult positivity or gross bleeding and stool consistency according to the method of Cooper et al.10) An asterisk indicates a significant difference in comparison to the control group (P < 0.05: non-parametric Dunnett’s multiple-comparison test). B, Level of lipid peroxide (TBARS) in the colonic mucosa of DSS-induced colitis mice. An asterisk indicates a significant difference in comparison to the control group (P < 0.05: Dunnett’s multiple-comparison test).

**Fig. 2.** Effects of *S. thermophilus* YIT 2001 and YIT 2084 on Anemia Resulting from DSS-Induced Colitis (Experiment 2).

A, Hemoglobin concentration in the blood of colitis mice. B, Hematocrit in the blood of colitis mice. Mice were fed on a control diet (Control), a diet containing 0.4% *S. thermophilus* YIT 2001 (YIT 2001), or a diet containing 0.4% *S. thermophilus* YIT 2084 (YIT 2084). An asterisk indicates a significant difference in comparison to the control group (P < 0.05: Dunnett’s multiple-comparison test).

In conclusion, *S. thermophilus* YIT 2001, which has high antioxidative activity, prevented oxidative injury to the colonic mucosa and improved the disease activity index in DSS-induced colitis mice. Furthermore, anemia following this colitis was reduced by the ingestion of this lactic acid bacterium. *S. thermophilus*
YIT 2001 may therefore be useful in the treatment of acute colitis.

Acknowledgments

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References


