Immediate Effect of *Lactobacillus acidophilus* on the Intestinal Flora and Fecal Enzymes of Rats and the *In Vitro* Inhibition of *Escherichia coli* in Coculture

O. Sreekumar and A. Hosono
Faculty of Agriculture, Shinshu University,
Nagano-Minamiminowa 399-4598, Japan

ABSTRACT

The in vitro role of *Lactobacillus acidophilus* was investigated to explore the potential to inhibit coliforms. A threefold concentrated cell-free extract from *L. acidophilus* SBT2074 could efficiently inhibit most of the tested Gram-positive and Gram-negative bacteria. Among the three strains of *L. acidophilus*, SBT2062, SBT2071, and SBT2074, only *L. acidophilus* SBT2074 showed this inhibitory property. These three strains were also tested in coculture with *Escherichia coli* 3544 in skim milk medium. The fermentation could result in complete inhibition of *E. coli* in 36 h. Short-term administration of *L. acidophilus* SBT2074 in rats with and without *E. coli* resulted in significant inhibition of coliforms and anaerobes. The *E. coli* infected rats regained the normal flora in the presence of lactic acid bacteria. The fecal enzyme β-glucuronidase activity was also decreased significantly when *L. acidophilus* SBT2074 was administered and was related to the decreased number of bacteria in the intestinal tract. The analysis of the small intestinal contents showed that the concentrations of coliforms in the duodenum, jejunum, and the ileum were significantly reduced by the administration of lactic acid bacteria. The effects are seen in a short period, suggesting that *L. acidophilus* SBT2074 fermentate may have clinical application for people suffering from gastrointestinal distress caused by coliforms.

(**Key words:** *Lactobacillus acidophilus*; β-glucuronidase; intestinal flora; *Escherichia coli*)

Abbreviation key: LAB = lactic acid bacteria.

INTRODUCTION

Lactic acid bacteria have traditionally been used in fermented foods. In fermented milk they produce lactic acid, various flavors combined with antimicrobial substances, which can inhibit natural contaminants and, occasionally, certain pathogens. The *Lactobacillus acidophilus* group of lactic acid bacteria (**LAB**) are widely used in fermented milk products, and the intake of these bacteria has beneficial effects on human health (6). LAB are known to produce a variety of antimicrobial substances such as organic acids, diacetyl, hydrogen peroxide, and bacteriocins. There is increased interest in the use of bacteriocins, produced by lactic acid bacteria, as food preservatives, because many of them are able to inhibit the growth of food-contaminating bacteria (14, 23).

The intestinal microflora comprise a complex ecosystem of a large variety of bacteria. These complex flora can produce negative and positive effects (9), and altering the intestinal microbiota in a beneficial way can improve the health of a host. Lactic acid bacteria have been considered potentially useful in this respect (20). When consumed, the transitting LAB in the gastrointestinal tract are capable of delivering enzymes and other substances into the intestine, which possibly help to control intestinal flora (1, 13). The dietary supplements of LAB as a preventative of colon cancer have received special attention in recent years. Some LAB have the ability to prevent DNA damage and mutations in vitro and in vivo (26). Carcinogenicity has always correlated with modification of gut bacterial activities. The consumption of *L. acidophilus* in animal experiments has shown a decreased activity of fecal enzymes such as β-glucuronidase, azoreductase, and nitroreductase (7, 8). The activities of these enzymes have been well correlated to the number of LAB in the intestine. Intestinal microflora can metabolize indirect-acting mutagens by means of specific microbial enzymes that reduce nitro and azo groups or hydrolyze glucuronides. Rowland et al. (17) have reported that the consumption of LAB could enable beneficial changes in cecal physiology and bacterial metabolic activity.

Several investigators have shown that *L. acidophilus* exerts antagonistic action against intestinal and foodborne pathogens and related organisms (11, 18). The present study explores the potential of three *L. acidophilus* strains in inhibiting both Gram-positive and Gram-negative bacterial strains. The effect was also

Received August 16, 1999.
Accepted November 17, 1999.
Corresponding author: A. Hosono; e-mail: ahosono@gipmc.shishu-u.ac.jp.

tested in coculture with *E. coli*, in skim milk medium. One of the selected strains was administered to rats to evaluate the immediate effect in controlling the intestinal microflora and the activities of β-glucosidase and β-glucuronidase. All studies were carried out on a short-term basis to evaluate the immediate effect on the host animal because most of the reported studies were carried out on a long-term basis. This study on the immediate effect is important, because colonization is a very complex process. Even the human isolate itself sometimes fails to colonize the human intestinal tract. Thus, if LAB could elicit an immediate effect in the host, they could generate the use of the same in clinical application.

MATERIALS AND METHODS

Bacterial Cultures

Three strains of *L. acidophilus*, SBT2071, SBT2074, and SBT2062, were obtained from Snow Brand Milk Company (Japan) and were maintained in MRS medium (Oxoid Ltd., Basingstoke, UK). Active cultures were prepared from the stock cultures, which were stored at –80°C. Lyophilized cells were prepared by harvesting cells from MRS medium grown for 24 h. The viability of the lyophilized cells was tested on MRS agar plates. Lyophilized cells of *E. coli* 3544 (from our stock cultures) were prepared in Oxoid nutrient broth No. 2 (Oxoid Ltd., Basingstoke, UK), and cells were harvested after 24 h of incubation at 37°C. The cells were lyophilized and viability was routinely checked on desoxycholate agar (Nissui, Tokyo, Japan).

Antibacterial Substance Production and Assay

The production of antibacterial substance from *L. acidophilus* strains was carried out in MRS medium. Cells were harvested by filtration after 24 h of growth and the pH of the filtrate was brought to 6.5 with 3 N NaOH. The filtrate was sterilized with sterile membrane filters (0.45 μM, Nacalai tesque, Japan) and lyophilized. A threefold concentrate was prepared by dissolving the lyophilized material in distilled water to one third of the original volume of the filtrate and used for the antimicrobial studies. Strains such as *Bacillus cereus, Bacillus subtilis, Enterobacter aerogenus, and Enterococcus fecalis* were propagated in Nutrient broth No. 2 (Oxoid, Basingstoke, UK), and *Listeria monocytogenus* in media containing glucose, 1 g/L; NaCl, 2 g/L; and tryptose, 20 g/L. Agar media plates were prepared by adding 15 g/L of agar (Ina agar, Nagano, Japan). Soft agars were prepared with 6 g/L of agar. All tester strains (from our stock culture, as indicated in Table 1) were prepared as fresh active cultures and cells of 1×10^7 (cfu/ml) were added to 5 ml of soft agar kept at 45°C, mixed well, and poured to the respective agar plate. Sterile paper disks (8 mm thick, Tokyo, Japan) were placed on the center of the agar plate and 20 μl of the filtrate was applied. Plates were incubated at 30 or 37°C according to the tester strain and observed for zones of inhibition.

Coculture in Skim Milk Medium

The active cultures of *L. acidophilus* were prepared by inoculating a small volume (5 ml) of sterile skim milk (10% skim milk medium, 115°C for 10 min) with the respective inoculum (1% vol/vol) and incubated at 37°C for 24 h. Each of the active cultures was inoculated at 1% (vol/vol) level to 10% sterile skim milk medium and incubated for 48 h at 37°C. For coculture studies, an active culture of *E. coli* 3544, was grown in Oxoid nutrient broth for 24 h at 37°C and inoculated at 1% (vol/vol) level to sterile skim milk medium. Samples were taken at different intervals and viable counts were determined by serial dilution on MRS agar and desoxycholate agar for *L. acidophilus* and *E. coli*, respectively. Initial and final pH of all samples was also measured.

Animals

Twenty-five male Wistar rats (6 wk old) with an average initial BW of 120 to 150 g (SLC, Kyoto, Japan), were placed in individual metabolic cages and housed in a room that was maintained at a constant temperature of 22 ± 2°C, a relative humidity of 60 ± 5%, and a 12-h light to dark cycle. Animal care was in accordance with the guidelines for Animal Experimentation of the Faculty of Medicine, Shinshu University. All rats were initially adapted for 5 d to a commercially available basal chow diet (Clea Japan Inc., Osaka, Japan).

Animals were divided into five groups of five rats each. The first group of five rats received basal diet containing lyophilized cells of *L. acidophilus* SBT2074 (final concentration 5×10^7 cfu/g diet). A second group was fed *E. coli* 3544 cells (final concentration 4×10^5 cfu/g diet) for 5 d, followed by *L. acidophilus* SBT2074 cells for the next 4 d. After administration of LAB, animals were put on normal diets for another 2 d. The third group was coadministered *L. acidophilus* SBT2074 and *E. coli* 3544 cells (as in group II) for 5 d, then the normal diet for the next two consecutive days. The fourth group was given lyophilized *L. acidophilus* SBT 2074 cells for 5 d and the fecal enzymes were analyzed, before, during, and after treatment. The fifth group was treated as control on normal diet. The fecal samples were collected before, during, and after treat-

Table 1. Inhibitory effects of fermented cell-free culture extracts of *Lactobacillus acidophilus* strains against Gram-positive and Gram-negative strains.[1]

	L. acidophilus SBT2062		*L. acidophilus* SBT2071		*L. acidophilus* SBT2074	
Tester strains	Glucose	Lactose	Glucose	Lactose	Glucose	Lactose
Escherichia coli						
3301	–	–	–	–	–	+++
3302	–	–	–	–	–	+++
3544	–	–	–	–	–	+++
K12	–	–	–	–	–	+++
Bacillus cereus						
3001	–	–	–	–	–	+++
3457	–	–	–	–	–	+++
IAM1029	–	–	–	–	–	+++
Enterobacter aerogenus						
3320	–	–	–	–	–	+++
Bacillus subtilis						
3312	–	–	–	–	–	+++
Enterococcus fecalis						
ATCC10100	–	–	–	–	–	+++
Listeria monocytogenes						
19111	–	–	–	–	–	+++
13932	–	–	–	–	–	+++

[1]– = No inhibition, + = relative inhibition. A threefold concentrate of the filtrate was used for the inhibition studies. *Bacillus* species were incubated at 30°C and others at 37°C.

ments. On the last day of the experimental period (group I and group V), rats were killed by cervical dislocation. The small intestine was divided into three regions: the duodenum, jejunum, and the ileum. These were separated and flushed with sterile prereduced (buffer kept overnight in an anaerobic jar, to remove the O_2 and saturate with CO_2) phosphate buffer (pH 6.98). Microbiological analysis was carried out as described earlier. For enzyme analysis, the cecum and the small intestine were removed and the entire contents transferred into prereduced phosphate buffer (pH 6.98). Rats were allowed to consume their diets and water ad libitum. The average daily consumption of food and water per rat was also calculated. The lyophilized cells were mixed with 2 g of normal diet and were fed each morning during the experimental period. After ensuring the complete consumption of cells (approximately 2 h), additional portions of normal diet were given. The BW of each rat was measured before separating the animals to individual cages and after completing the treatments.

Fecal Microbial Analysis

All fecal samples were collected fresh by gently squeezing the rectal area of the rat. The fecal pellets were immediately placed in tubes kept in anaerobic jars and the analysis was carried out within 30 to 60 min of collection. Anaerobic conditions were maintained as far as possible during the analysis. Following homogenization, a series of 10-fold dilutions of the specimens was made in a prereduced sterile phosphate buffer. Triplicate plates were made of each sample in plate count agar (Oxoid, Basingstoke, UK) for total anaerobes and total aerobes, desoxycholate agar for coliforms, and MRS agar for LAB. Plates of anaerobe and LAB were incubated anaerobically in an anaerobic chamber (BBL Gas Pak anaerobic jars, Becton Dickinson Co., Franklin Lakes, NJ) for 3 d at 37°C. Plates for the enumeration of aerobic organisms and coliforms were incubated at 37°C for 2 d.

Fecal Enzyme Assays

Fresh fecal samples were collected by gently squeezing the rat's rectal region to release the fecal pellets. The samples were collected in sterile tubes kept overnight in an anaerobic chamber (BBL, Becton Dickinson).

β-Glucuronidase Assay

Fresh fecal samples were suspended in cold prereduced 0.1 *M* potassium phosphate buffer (pH 7.0). The fecal suspension was homogenized and was disrupted by sonication with a sonicator (Sonicator USP 300, Shimadzu, Kyoto, Japan) for 3 min at 4°C. The samples were centrifuged at 500 × *g* for 15 min. The supernatant was collected and immediately used for the enzyme assay. The enzyme reaction was run at 37°C (pH 6.8) as described by Goldin and Gorbach (7). Briefly, the total volume of reaction mixture was 1 ml, containing a final concentration of 0.02 *M* potassium phosphate buffer, 0.1 m*M* EDTA, 1 m*M* phenolphthalein-β-D-glucuronide (Sigma, St. Louis, MO) and 0.1 ml of fecal

extract. The reaction was stopped by adding 5 ml of 0.2 *M* glycine buffer (pH 10.4) containing 0.2 *M* NaCl. Readings were taken at 540 nm. The amount of phenolphthalein released was determined by comparison with a standard phenolphthalein curve and expressed as specific activity (μmol/mg of protein per 30 min).

β-Glucosidase Assay

The fecal extract was prepared as above. The enzyme reaction was run at 37°C (pH 7.4) in a total volume of 1 ml containing a final concentration of 0.1 *M* potassium phosphate buffer, 1 m*M* nitrophenyl-β-D-glucoside (Sigma) and 0.2 ml of fecal extract. The reaction was run for 30 min at 37°C. The reaction was stopped by the addition of 5 ml of 0.01 *M* NaOH. Readings were taken at 420 nm. The amount of nitrophenol released was determined by comparison with a standard nitrophenol curve and expressed as μmol/mg of protein per 30 min.

Protein Determination

Protein concentrations in the fecal extracts were determined by the method of Lowry et al. (12) with bovine serum albumin as standard.

Statistical Analyses

Results obtained were subjected to Student's t-test using SPSS (21), version 6.0. Standard error and level of significance were calculated and compared to control animals or with the values of before administration (0 d) of the respective group.

RESULTS AND DISCUSSION

Lactobacillus acidophilus SBT2071, SBT2074, and SBT2062 were tested for the antibacterial compound production against both Gram-positive and Gram-negative bacteria. The culture filtrates (MRS glucose) of these three strains failed to produce any inhibitory compounds against the indicator strains (*E. coli, B. cereus, B. subtilis, E. aerogenus, E. fecalis* and *L. monocytogenus*) even after a threefold concentration (Table 1). When these *L. acidophilus* strains were grown in lactose MRS media, strain SBT2074 was able to inhibit all tested indicator strains with the threefold concentrate, but the other two strains failed to produce any inhibitory compounds. An increased bacteriocin production was also observed when *Lactobacillus gasseri* SBT10239 strain was grown in lactose MRS medium (O. Sreekumar and A. Hosono, 2000, unpublished). Various reports show that temperature, pH, and carbon source also affect bacteriocin production (2, 3, 23).

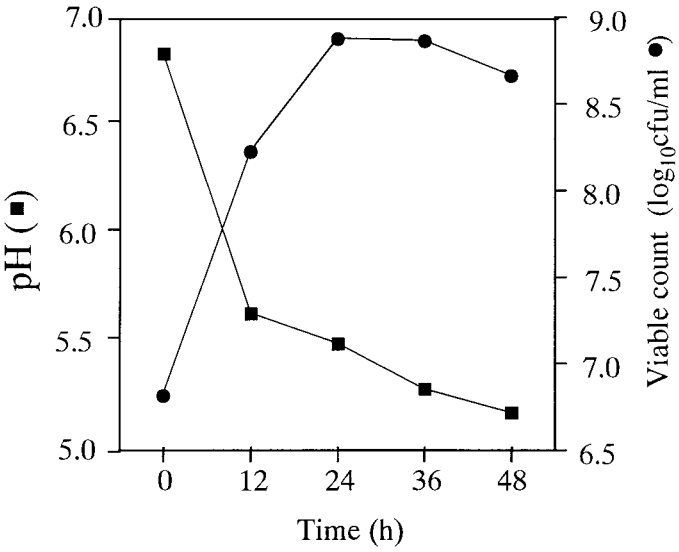

Figure 1. Growth pattern of *Escherichia coli* 3544 strain in skim milk medium.

To further confirm the in vitro inhibitory role, these three strains of *L. acidophilus* were grown as pure and coculture with *E. coli* in skim milk medium. In Figure 1, the growth pattern of *E. coli* 3544 shows that the stationary phase was reached in 24 h, and the pH dropped to 5.3. A two-log increase of cells was observed in 24 h.

Figures 2, 3, and 4 show the growth pattern of *L. acidophilus* in pure and coculture with *E. coli* 3544. The viable count and pH were measured. Strain SBT2071 showed a similar growth pattern when grown in pure culture and coculture. The number of viable cells was higher in coculture than in pure culture (Figure 2). In fact, *E. coli* failed to grow in coculture and the number of viable counts was constant until 24 h, after which the viability decreased sharply. This was probably caused by the acid production along with other inhibitory compounds produced during the fermentation of milk by LAB. After 48 h of fermentation none of the *E. coli* cells was viable.

The growth pattern and acid production of SBT2062 were the same, irrespective of the culture condition used. The log phase, stationary phase, and the decrease in pH (Figure 3) were the same in pure culture and coculture. The viable count of *E. coli* was decreased to 4 log (cfu) and to zero in 24 and 36 h, respectively. This can be attributed to the faster growth rate of SBT2062, along with the reduction in pH from 6.5 to 4.2 in 12 h of fermentation. A decline in viable cells of SBT2062 was observed after 36 h of fermentation, which was consistent in pure culture and coculture. The same

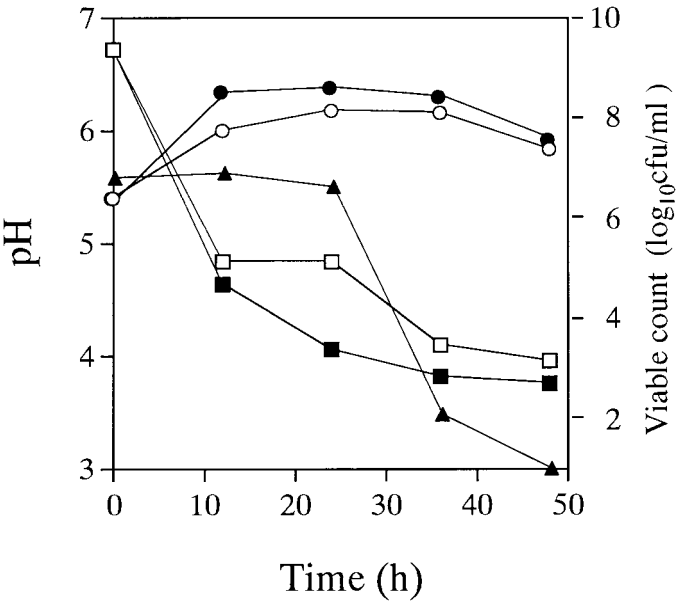

Figure 2. Growth of *Lactobacillus acidophilus* SBT2071 in pure culture and coculture with *Escherichia coli* 3544 in skim milk medium. □ ■ pH; ○ ● *L. acidophilus* SBT2071; △, *E. coli* 3544; closed symbol, coculture; open symbol, pure culture.

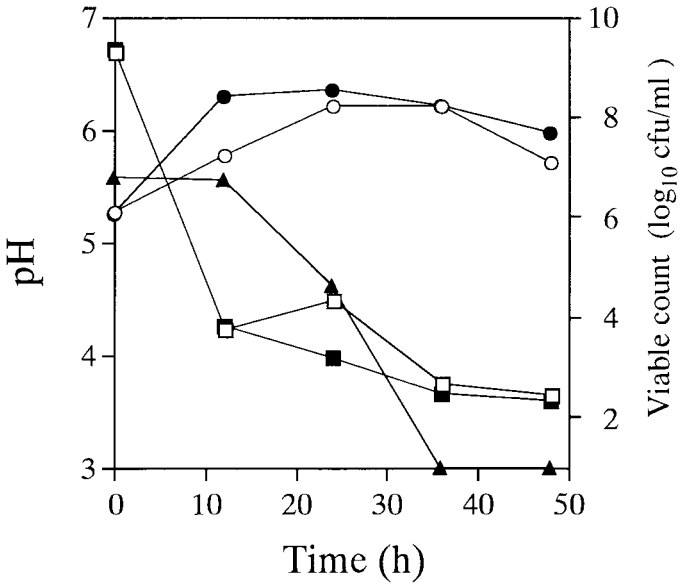

Figure 4. Growth of *Lactobacillus acidophilus* SBT2074 in pure culture and coculture with *Escherichia coli* 3544 in skim milk medium. □ ■ pH; ○ ● *L. acidophilus* SBT2071; △, *E. coli* 3544; closed symbol, coculture; open symbol, pure culture.

strain of *L. acidophilus* SBT2062 also inhibited some pathogens in coculture (22).

Strain SBT2074, which was the only strain that showed production of antibacterial substance in MRS lactose media, had a different pattern of growth in pure culture and coculture. The log phase was up to 24 h in pure culture, whereas it took only 12 h in coculture (Figure 4). The acid production, as represented by the decrease in pH, showed a similar pattern in both pure culture and coculture. The decline in viable count of LAB was significant in pure culture after 36 h of fermentation. The decrease in the viable count of *E. coli* was proportional to the increased growth of *L. acidophilus* SBT 2074. The acid produced with the antimicrobial substance during growth may be responsible for the sharp decline in the *E. coli* count. Hosono et al. (11) have also reported the production of antibiotic-like substances by *L. acidophilus* IFO3205, which could repress the growth of *E. coli*. Referring to Table 1, *L. acidophilus* SBT2074 produced the inhibitory compound when grown in lactose medium. Milk, a rich source of lactose, also might have contributed to the inhibition of *E. coli*. Strain SBT2074 was used for further in vitro studies using rat as a model.

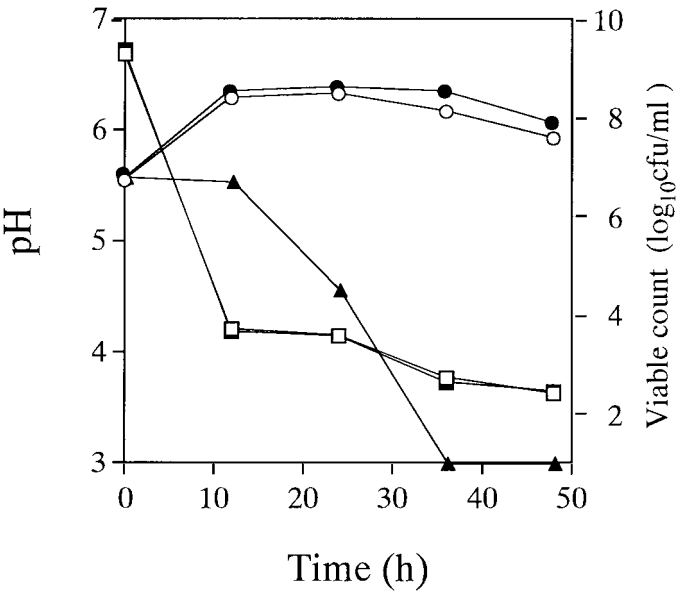

Figure 3. Growth of *Lactobacillus acidophilus* SBT2062 in pure culture and coculture with *Escherichia coli* 3544 in skim milk medium. □ ■ pH; ○ ● *L. acidophilus* SBT2062; △, *E. coli* 3544, closed symbol, coculture; open symbol, pure culture.

Animals were divided and treated as described in materials and methods. There were no significant differences in feed intake, water consumption, and weight gain among the groups to the control group (data not shown). Figure 5 shows the effect on the consumption of *L. acidophilus* on the fecal microflora of the rat. Viability of *L. acidophilus* was checked routinely before administration. During the intake of LAB the number of coliforms ($P < 0.05$) along with total aerobes ($P < 0.001$) and anaerobes ($P < 0.05$) decreased significantly

versus the 0-d count. A significant increase in total LAB count was observed, probably because the consumed LAB were excreted viable or because this strain colonizes the intestinal tract. After treatment was stopped and the diet shifted to normal food, a significant ($P < 0.001$) decrease in coliforms was observed, but the aerobic and anaerobic count remained constant. This implies that strain SBT2074 resulted in significant in vitro inhibition of coliforms along with the aerobes and anaerobes. The consumption of *L. acidophilus* milk has been reported to have changed the fecal flora of humans (1). The strain SBT2074 might have colonized the intestine and influenced the intestinal flora. The in vitro inhibition of *E. coli* and the in vitro inhibition of coliforms were thus well related.

The second group of animals was initially fed with lyophilized *E. coli* cells for 5 d, followed by *L. acidophilus* SBT2074 cells (Figure 6). As expected, the *E. coli* 3544 administration has resulted in increased numbers of coliforms ($P < 0.001$) and anaerobes ($P < 0.05$). The LAB count was not significantly affected during the *E. coli* 3544 administration. But administration of

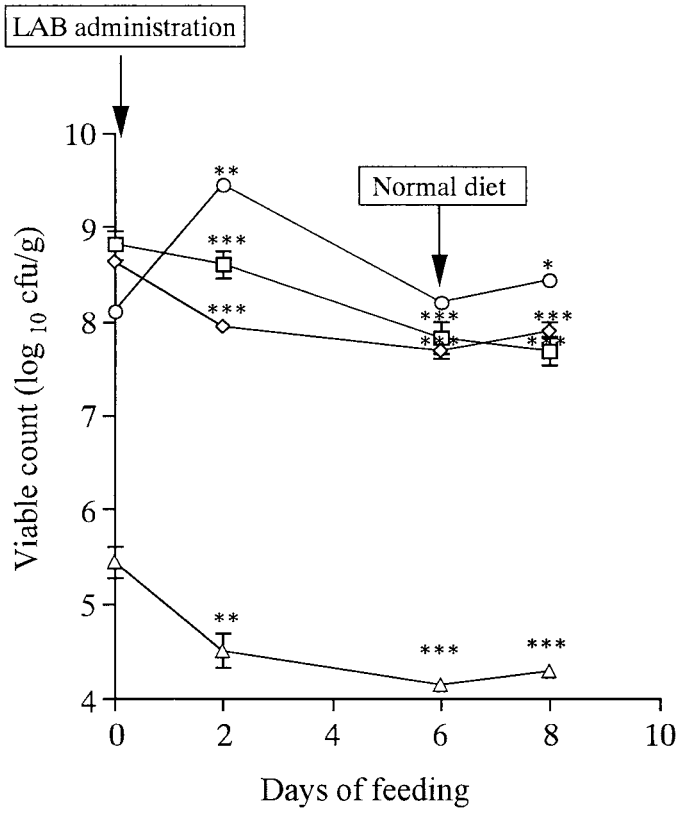

Figure 5. Effect of feeding *Lactobacillus acidophilus* SBT2074 cells on the rat fecal bacterial count. (n = 5), □ Aerobes; ◇ anaerobes; ○ lactic acid bacteria; △ coliforms. *$P < 0.01$; **$P < 0.05$; ***$P < 0.001$ versus 0 d. LAB = Lactic acid bacteria.

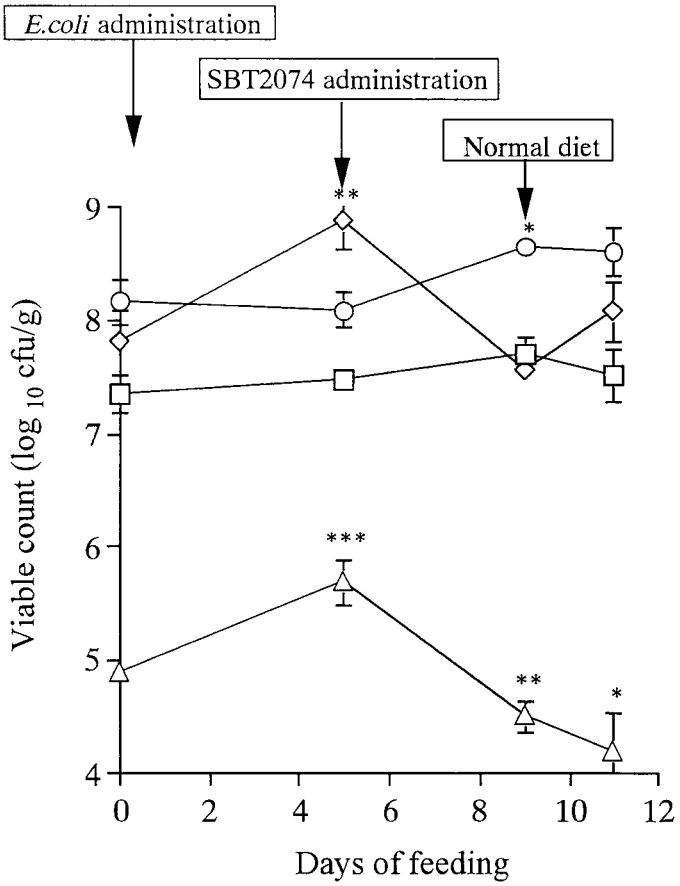

Figure 6. Effect of *Escherichia coli* 3544 administration followed by *Lactobacillus acidophilus* SBT2074 administration on the fecal bacterial count. (n = 5), □ Aerobes; ◇ anaerobes; ○ lactic acid bacteria; △ coliforms. *$P < 0.01$; **$P < 0.05$; ***$P < 0.001$ versus 0 d.

LAB decreased ($P < 0.05$) the coliform and total anaerobe counts, without much variation in total aerobes. The LAB count also increased significantly ($P < 0.01$) and remained constant even after 2 d of normal diet. The coliform count after 2 d of normal diet was significantly ($P < 0.01$) lower than 0-d count. This indicates that some significant changes in the intestinal flora occurred in the presence of *L. acidophilus* SBT2074, which can probably protect for a longer period by colonizing the intestinal tract.

The coadministration of *E. coli* 3544 and *L. acidophilus* SBT2074 was carried out in a third group of animals (Figure 7). The coadministration resulted in significant ($P < 0.01$) decrease in coliforms and one-log (cfu) increase in lactic acid bacterial count ($P < 0.05$), suggesting that, even in the presence of coliforms, strain SBT2074 was able to colonize or positively influence the intestinal tract and bring the intestinal microflora back to normal. After treatment, the coliform level was significantly lower ($P < 0.01$) than the 0-d

count, but an increase in aerobic count ($P < 0.05$) was observed. Changes in the composition of microflora in rats by yogurt bacteria has also been reported (4). Thus, this study confirms that coliforms can be inhibited in vitro, in coculture and in vitro of animals by *L. acidophilus* SBT2074 strain. Moreover, strain SBT2074 resulted in an immediate effect in the host, and the effect persisted even after the LAB administration was terminated. The consumption of strain SBT2074 through fermented milk or milk with viable cells may be able to control the gastrointestinal disorders caused by coliforms.

The concentrations of β-glucosidase and β-glucuronidase were measured in the feces of rats treated with *L. acidophilus* SBT2074 strain (Figure 8). The specific activity of β-glucuronidase rapidly decreased ($P < 0.001$) when the rats were administered with strain SBT2074. Goldin and Gorbach (8) reported that the consumption of LAB have significantly reduced the activities of fecal enzymes, which are implicated in colon carcinogenesis. But the increase in the activity of β-glucoidase was observed after the administration of LAB. The high levels of β-glucosidase activity can be correlated to the increased number of lactic acid bacteria (Figure 5), which have high levels of β-glucosidase activity in comparison with other members of the gut microflora (19). The β-glucuronidase has wide substrate specificity and can hydrolyze many different glucuronides. Cleaving of the β-glucuronide bond by the gut microflora may also release biologically active aglycones, such as diethylstilbesterol-β-glucuronide (5) and N-hydroxyfluorenylacetamide-β-glucuronide (10, 25), which are potentially carcinogenic. The change in microflora is responsible for the change in β-glucuronidase activity (16). The β-glucuronidase was reported to be produced by *E. coli*, Bacterioides and some obligate anaerobes (24), and, from our results, it is reasonable to conclude that the decreased activity of β-glucuronidase was caused by the decreased number of anaerobes and coliforms.

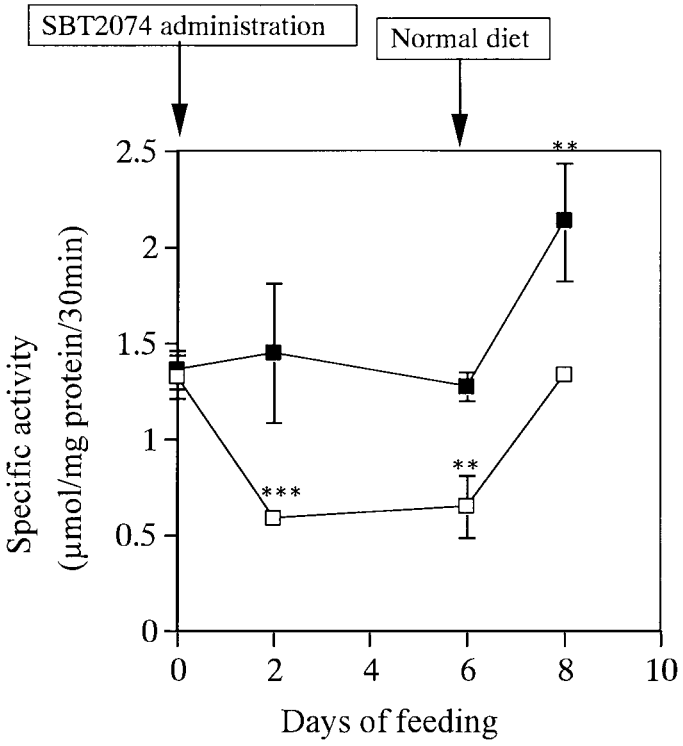

Figure 8. Effect of feeding *Lactobacillus acidophilus* SBT2074 cells on specific activity of β-glucosidase (■) and β-glucuronidase (□) in the feces of rats. Specific activity was expressed as the μmol of nitrophenol or phenolphthalein released per mg of fecal protein in 30 min, n = 5, bar line represent the standard error. $^{**}P < 0.05$; $^{***}P < 0.001$ versus 0-d activity.

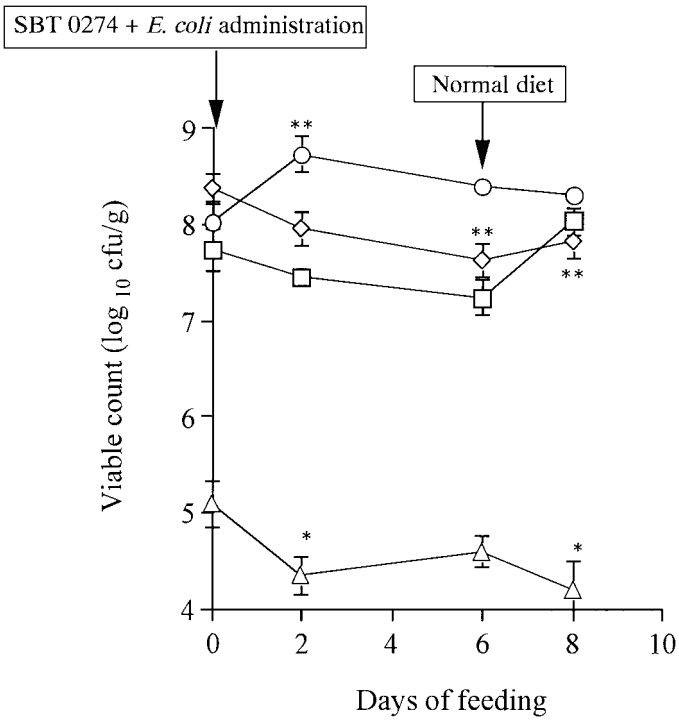

Figure 7. Effect of *Lactobacillus acidophilus* SBT2074 and *Escherichia coli* coadministration in the fecal microflora of rat. (n = 5), □ Aerobes; ◇ anaerobes; ○ lactic acid bacteria; △ coliforms. $^{*}P < 0.01$; $^{**}P < 0.05$; $^{***}P < 0.001$ versus 0 d.

The small intestinal contents were analyzed to locate the area where the major changes in microflora occurred because of the administration of LAB (Figure 9). There was a significant difference in coliform counts in the duodenum ($P < 0.001$), jejunum ($P < 0.05$), and the ileum ($P < 0.05$). The anticarcinogenic effects of *L. acidophilus* with experimental animals have shown that incorporation of *L. acidophilus* in their diet significantly reduced the fecal *E. coli* counts (15). A sig-

nificant increase in LAB ($P < 0.01$) count was observed in the jejunum. Significant increase in anaerobes were observed in the duodenum ($P < 0.05$), but a sharp decrease in anaerobes was seen in the jejunum ($P < 0.01$). The low concentrations of total microflora in the duodenum can be correlated to the continuous secretion of bile from bile duct into the duodenum, since rats lack the gall bladder (24).

The same lowered β-glucuronidase activity ($P < 0.05$) was observed in the cecal content of LAB-administered rats as the control group (Figure 10). But the low levels of activities of these enzymes observed in the small intestine can be related to the low counts in coliforms and anaerobes (Figure 9). The largest microbial community was present in the large intestine (24), which accounts for the high β-glucuronidase activity in the cecum. No significant difference in the levels of β-glucosidase was observed in the small intestine and the cecum.

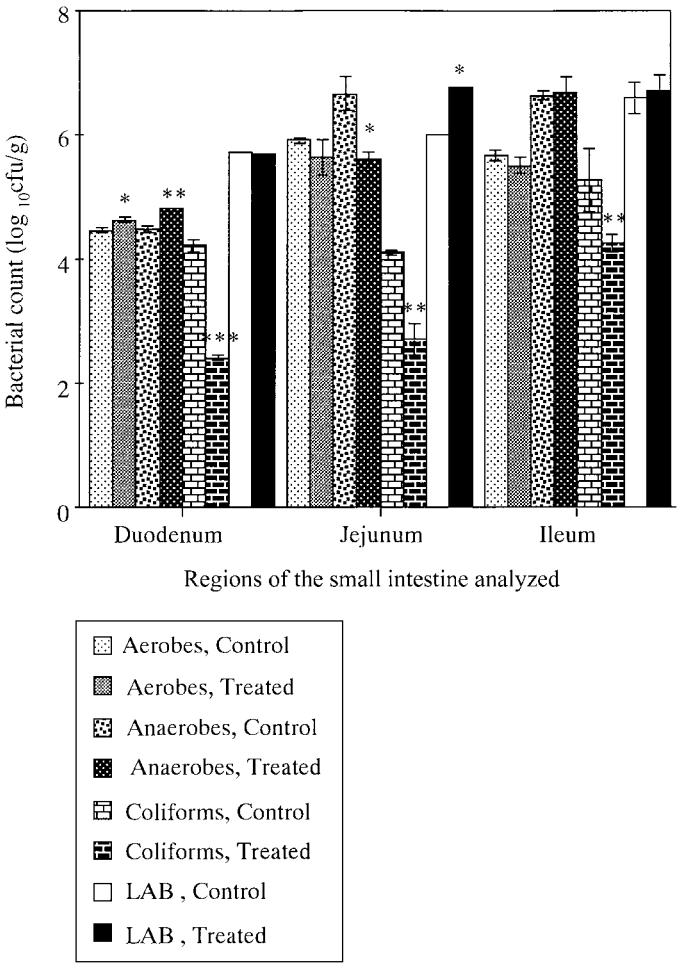

Figure 9. Effect of *Lactobacillus acidophilus* SBT2074 administration on the small intestinal microflora compared with the control rats (n = 3). Duodenum, starting region of small intestine; jejunum, middle region of the small intestine; ileum, hind region of small intestine entering the cecum. Values are with standard error; *$P < 0.01$; **$P < 0.05$; ***$P < 0.001$.

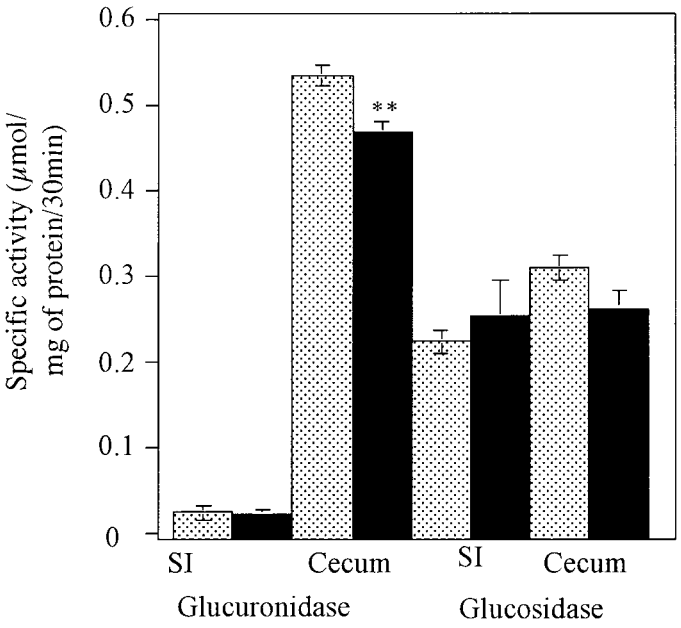

Figure 10. Concentrations of fecal enzymes in the cecum and small intestines of lactic acid bacteria-treated and control rats. Control rats (light shade) and *Lactobacillus acidophilus* treated (dark shade). (n = 3) SI = Small intestine, **$P < 0.05$.

CONCLUSIONS

Lactobacillus acidophilus SBT2074 was able to inhibit coliforms in both fermented milk and in the intestines of rats. The immediate effect was due to the inhibition by this strain, thus the use of this strain in fermented milk products is of equal importance. Moreover, consumption of strain SBT2074 as viable cells together with milk or as a fermented product may result in controlling the intestinal flora and fecal enzymes. This strain deserves attention in clinical application for people suffering from gastrointestinal disorders caused by coliforms. Further studies need to be carried out in human subjects to confirm this view.

ACKNOWLEDGMENTS

The authors are thankful to M/s Snow Brand Milk Co., Tokyo, Japan for the financial help and supplying the bacterial cultures.

REFERENCES

1 Ayebo, A. D., I. A. Angelo, and K. M. Shahani. 1980. Effect of ingesting *Lactobacillus acidophilus* milk upon fecal flora and enzyme activity in humans. Milchwissenschaft 35:730–733.

2 Barefoot, S. F., and T. Klaenhammer. 1983. Detection and activity of lactacin B, a bacteriocin produced by *Lactobacillus acidophilus*. Appl. Environ. Microbiol. 45:1808–1815.
3 Dave, R. I., and N. P. Shah. 1998. Characteristics of bacteriocin produced by *Lactobacillus acidophilus* LA-1. Int. Dairy J. 7:707–715.
4 Djouzi, Z., C. Andrieux, M. C. Degivry, C. Bouley, and O. Szylit. 1997. The association of yogurt starters with *Lactobacillus casei* DN 114.001 in fermented milk alters the composition and metabolism of intestinal microflora in germ-free rats and in human flora-associated rats. J. Nutr. 127:2260–2266.
5 Fisher, L. J., P. Millburn, and R. L. Smith. 1966. The fate of ^{14}C stilbesterol in the rat. Biochem. J. 100:69–76.
6 Gilliland, S. E. 1990. Properties of fermented milk. FEMS Microbiol. Rev. 87:175–188.
7 Goldin, B. R., and S. L. Gorbatch. 1976. The relationship between diet and rat fecal bacterial enzymes implicated in colon cancer. J. Natl. Cancer Inst. 57:371–375.
8 Goldin, B. R., and S. L. Gorbach. 1984. Alterations of the intestinal microflora by diet, oral antibiotics and lactobacillus: Decreased production of free amines from aromatic nitrocompounds, azodyes and glucuronides. J. Natl. Cancer Inst. 73:689–695.
9 Gorbach, S. L. 1986. Function of the normal human microflora. Scand. J. Infect. Dis. 49:17–30.
10 Grantham, P. H., R. E. Horton, and E. K. Weisburger. 1970. Metabolism of the carcinogen N-2-fluroenylacetamide in germ-free and conventional rats. Biochem. Pharmacol. 19:163–172.
11 Hosono, A., K. Yatsuki, and F. Tokita. 1977. Isolation and characterization of an inhibitory substance against *Escherichia coli* produced by *Lactobacillus acidophilus*. Milchwissenschaft 32:727–730.
12 Lowry, O. H., N.J.N. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with folin phenol reagent. J. Biol. Chem. 193:265–275.
13 Mallet, K., and I. R. Rowland. 1988. Factors affecting the gut microflora. Pages 347–355 *in* Role of the Gut Flora in Toxicity and Cancer. I. R. Rowland, ed., Academic Press, London.
14 Nettles, C. G., and S. F. Barefoot. 1993. Biochemical and genetic characteristics of bacteriocins of food-associated lactic acid bacteria. J. Food Prot. 56:338–356.
15 Muralidhara, K. S., G. G. Sheggeby, P. R. Elliker, D. C. England, and W. E. Sandine. 1977. Effect of feeding lactobacilli on the coliform and lactobacillus flora of intestinal tissue and feces from piglets. J. Food Prot. 40:288–295.
16 Reddy, B. S., J. H. Weisburger, and E. L. Wynder. 1974. Fecal bacterial β-glucuronidase: control by diet. Science 183:416–417.
17 Rowland, I. R., C. J. Rumney, J. T. Coutts, and L. C. Lievense. 1998. Effect of *Bifidobacterium longum* and inulin on gut bacterial metabolism and carcinogen-induced aberrant crypt foci in rats. Carcinogenesis 19:281–285.
18 Sabine, D. B. 1963. An antibiotic like effect of *Lactobacillus acidophilus*. Nature 199:811.
19 Saito, Y., T. Takano, and I. R. Rowland. 1992. Effect of soybean oligosaccharides on the human gut microflora in *in vitro* cultures. Microb. Ecol. Health Dis. 5:105–110.
20 Sanders, M. E. 1993. Summary of conclusions from a panel of experts on health attributes of lactic cultures: Significance to fluid milk products containing cultures. J. Dairy Sci. 76:1819–1828.
21 SPSS for Windows, User's Guide: Basic System, release 6.0. 1993. SPSS Inc., Chicago, IL.
22 Takiguchi, R., E. Mochiguki, Y. Suzuki, I. Nakajima, and Y. Benno. 1997. Inhibitory effects of *Lactobacillus acidophilus* SBT2062 and *Bifidobacterium longum* SBT2928 on harmful intestinal bacteria. J. Intest. Microbiol. 11:11–17.
23 Tagg, J. R., A. S. Dajani, and L. W. Wannamaker. 1976. Bacteriocins of Gram-positive bacteria. Bacteriol. Rev. 40:722–756.
24 Tannock, G. W. 1997. Normal microbiota of the gastrointestinal tract of rodents. Pages 187–215 *in* Gastrointestinal Microbiology. I. R. Mickie, B. A. White, and A. E. Isaacson, ed. Chapman and Hall, New York, NY.
25 Weisburger, J. H., P. H. Grantham, and R. E. Horton. 1970. Metabolism of the carcinogen N-hydroxy N-2 fluroenylacetamide in germ-free rats. Biochem. Pharmacol. 19:151–162.
26 Wollowski, I., S. Ji, A. T. Bakalinsky, C. Neudecker, and B. L. Pool-Zobel. 1999. Bacteria used for the production of yogurt inactivate carcinogens and prevent DNA damage in the colon of rats. J. Nutr. 129:77–82.