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Comparative Evaluation of Probiotic Activities of *Bifidobacterium longum* MK-G7 with Commercial Bifidobacteria Strains

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Abstract This study was conducted to compare probiotic activities and physiological functions of *Bifidobacterium longum* MK-G7 with several commercial and type strains of bifidobacteria. *Bif. longum* MK-G7 showed the highest acid tolerance against HCl and acetic acid, whereas *Bif. infantis* Y-1 showed the lowest acid tolerance and more than 4 log cycles of viable cell count decreased due to acid injury. Viable cell counts of bifidobacteria strains decreased more than 1.5 log cycles owing to oxygen toxicity, with the exception of *Bif. longum* MK-G7, *Bif. infantis* Y-2, *Bif. longum* Y-3, *Bif. longum* Y-6, and *Lactobacillus rhamnosus* Y-7. Among the bifidobacteria strains tested, *Bif. infantis* Y-2, *Bif. longum* Y-3, *Bif. longum* Y-6, and *Bif. longum* RD-13 showed the highest bile tolerance, whereas *Bif. longum* MK-G7 showed a medium level of bile tolerance. Only *Bif. longum* MK-G7 showed much higher antibiotic resistance against both tetracycline and penicillin-G in the MIC (minimum inhibitory concentration) level of 24.8 mg/l and 0.52 mg/l, respectively. *Bif. longum* MK-G7 showed a higher degree of *in vitro* cholesterol assimilation, followed by *Bif. breve* ATCC 15700 and *Bif. longum* RD-13. *Bif. longum* MK-G7, *Bif. lactis* Y-4, *Bif. longum* Y-6, and *Bif. bifidum* ATCC 29539 showed more than 80% of anti-mutagenicity against NQO (4-nitroquinoline-1-oxide). Since the production of cytokines such as TNF (tumor necrosis factor)-α and IL (interleukin)-6, and NO (nitric oxide) in the macrophage cell line Raw 264.7 cells increased as *Bif. longum* MK-G7 cell concentration increased, it was suggested that *Bif. longum* MK-G7 is able to enhance immunopotentiating activity *in vitro*. When freeze-dried *Bif. longum* MK-G7 was administered to mice at the dose of 1, 2, 4, and 6 g/kg of body weight, all of the mice survived in all feeding groups, proving the GRAS (generally recognized as safe) status of *Bif. longum* MK-G7. When fermented milk containing *Bif. longum* MK-G7 was administered to human volunteers, viable cell count of total bifidobacteria and anaerobes in the feces increased up to 0.5 log cycles more than before the administration. In particular, *Bif. longum* MK-G7 inhibited the growth of *Bacteroides* at the level of 1.0–1.5 log cycles.

Key words: *Bifidobacterium longum* MK-G7, probiotic activity, physiological functionality, Korean origin

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A probiotic microorganism has been defined as a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance. Some selection criteria must be met before a probiotic can be described as being useful [16, 17]. Normal gut microflora consists of microorganisms which live in a stable relationship with the host. The gut microflora have an effect on the development of the intestinal structure of the host and on the defense systems such as immune systems and microbial interference with resistance to intestinal infections [8, 20]. The composition of the microbial flora in the large intestine and feces of different age groups may differ [34]. Bifidobacteria remain as a major component of the cultivatable microflora and may play significant roles in the intestinal tracts of humans and animals. They produce organic acids which inhibit the growth of undesirable bacteria, and stimulate intestinal peristalsis. Their consumption also influences the metabolism of gut microflora, and some reports have indicated the possible value of bifidobacteria in improving the nutrition of humans. This potentially beneficial role of bifidobacteria in the intestinal tracts has led to their suggested use as dietary adjuncts in combination with their growth-promoting substances. Consequently, fermented milk products containing bifidobacteria may improve the nutritional and health values of the weaning diet [1, 2,

3, 14, 22, 27, 40, 41]. Herein, we report the characterization of newly isolated *Bif. longum* MK-G7 of the Korean-origin with respect to its high survival under several stress conditions and successful colonization during the administration period on human trial.

MATERIALS AND METHODS

Test Cultures and Enzymatic Activities

The isolation and description of *Bif. longum* MK-G7 were previously reported [26] and test cultures used are listed in Table 1. For preservation and storage, test cultures were streaked and incubated on BS agar medium. Typically, pure colonies were taken from the plates and serially transferred three times into MRS soft agar. For the measurement of enzyme activities, cell pellets were harvested from cultures and washed twice with distilled water. Cells were suspended in 10 ml phosphate buffer (pH 6.5) and sonicated under ice. The supernatant after centrifugation was used for measuring enzyme activities. The reaction mixture consisted of 100 µl of 10 mM substrate, 100 µl of 0.1 M phosphate buffer (pH 6.5), and 50 µl of enzyme solution (100 µl for β-glucosidase), and was incubated at 40°C for 10 min (20 min for β-glucosidase). Then, 1,000 µl of 0.5 M sodium carbonate was added to stop the reaction and the absorbance at 400 nm was measured within 15 min.

Organic Acid Production and Acid Tolerance Against HCl and Acetic Acid

Test cultures were centrifuged at 3,000 rpm for 10 min. One-ml of supernatant was filtered through a 0.45 µm syringe filter (Millex-HA, Millipore, U.S.A.) and analyzed with HPLC (Waters 510, Millipore, U.S.A.). In addition to this, test cultures were incubated in modified TP (MTP) medium [24] for 24 h and centrifuged. The cell pellet was serially diluted and plated on neutral MTP medium (pH 7.0) and acid MTP medium, respectively. Test cultures were incubated in an anaerobic glove box (Forma Scientific, U.S.A., H_2:CO_2:N_2=5:15:80) at 37°C for 48 h. Acid MTP medium was adjusted to pH 4.8 using acetic acid and HCl. Acid tolerance was compared by measuring the absorbance at 600 nm of MRS broth, which contained 0.05% L-cysteine · HCl, and was incubated with a 1% inoculum size under anaerobic cultural condition for test cultures. Finally, acid tolerance was regarded as the ratio of viable cell count on acid MTP medium to viable cell count on neutral MTP medium.

Table 1. Test culture strains used in the experiment.

Test culture strains	Original sources
Bif. infantis Y-1	Commercial
Bif. infantis Y-2	Commercial
Bif. longum Y-3	Commercial
Bif. lactis Y-4	Commercial
Bif. longum Y-5	Commercial
Bif. longum Y-6	Commercial
Lb. rhamnosus Y-7	Commercial
Bif. adolescentis Y-8	ATCC 15706
Bif. bifidum Y-9	ATCC 29539
Bif. breve Y-10	ATCC 15700
Bif. infantis Y-11	ATCC 15697
Bif. longum Y-12	ATCC 15707
Bif. longum MK-G7	Maeil Dairy
Bif. longum RD-13	Maeil Dairy

Oxygen Tolerance

Test cultures were incubated in MTP broth for 24 h and centrifuged. The cell pellet was serially diluted and plated on MTP medium. Then, test cultures were incubated aerobically at 37°C for 0 and 20 h. Thereafter, plates were cultivated in an anaerobic incubator for 48 h. Oxygen tolerance was regarded as the ratio of viable cell count on aerobically cultivated cultures to viable cell count on anaerobically cultivated cultures.

Antibiotic Resistances

Various concentrations of tetracycline and penicillin-G were mixed in MTP medium. Activated test cultures were cultivated at 37°C for 48 h using the replica method in an anaerobic glove box. The MIC (minimum inhibitory concentration) was described as the minimum antibiotic concentration in which test cultures could not proliferate at all.

Bile Tolerance and Cholesterol Assimilation

Test cultures were cultivated in MTP broth for 24 h and centrifuged. The cell pellet was plated on MTP medium which contained 0.25% ox-bile (Oxoid, U.K.) and cultivated anaerobically for 72 h. Bile tolerance was also compared by measuring the absorbance at 600 nm of MRS broth (pH 7.0, containing 0.05% L-cysteine · HCl) which contained 0, 0.3, 0.5, and 1.0% of bile salt No. 3 (Difco, U.S.A.) and incubated with 1% inoculum size under anaerobic cultural condition for test cultures. Bile tolerance was regarded as the ratio of viable cell count in ox-bile (Oxoid, U.K.) containing MTP medium to viable cell count on normal MTP medium. In addition, as performed according to Danielson *et al.* [7], test cultures were incubated in MRS medium containing polyoxyethanyl-cholesterol (Sigma, U.S.A.) at the concentration of 360 µg/ml for 24 h. Then, 0.3% bile acid was added into the medium in order to investigate the effect of bile acid. The absorbance at 550 nm was measured and cholesterol reduction was calculated as follows: Cholesterol reduction (%)=[(Cholesterol added-Cholesterol left)/Cholesterol added]×100.

Antimutagenicity and Immunopotentiating Activities

The antimutagenicity of test culture was measured by the preincubation test described by Maron and Ames [31]. In the *Salmonella typhimurium* reversion assay, NQO was used as a direct mutagen which did not need S9 mixture for the activation. Antimutagenic activity was calculated as follows: Antimutagenicity (%)=100-[(No. of revertants by preincubation mixture-spontaneous revertants)/(No. of revertants by mutagen-spontaneous revertants)]×100. In addition to this, the production of TNF (tumor necrosis factor)-α and IL (interleukin)-6 were measured by ELISA (enzyme-linked immunosorbent assay) according to Dong *et al.* [12]. Heat-killed *Bif. longum* MK-G7 were co-cultured with a murine macrophage Raw 264.7 cell line at the concentration of 5×10^5 cells/ml in a 96-well flat-bottomed tissue culture plate. The production of NO (nitric oxide) was determined by measuring the optical density at 550 nm.

Acute Toxicity and Human Clinical Test

Male ICR mice (5-6 weeks) were used for the acute toxicity test. Five mice each were grouped and administered chow diet for 5 days in order to adapt the mice to experimental condition. Then, feeding was finished before 3 h of administration of *Bif. longum* MK-G7. Freeze-dried *Bif. longum* MK-G7 (8×10^{11} cfu/g) was suspended in distilled water and administered orally at the concentration of 1, 2, 4, and 6 g/kg of body weight everyday. Distilled water was used as control. Variation of body weight, mobility, and appearances were measured after 7 days of administration, and the mice were sacrificed for inspection of intestinal tracts. In addition, *Bif. longum* MK-G7 and *Bif. longum* Y-5 were inoculated in milk base in order to make fermented milks, and cultivated aerobically. Eight volunteers participated in the clinical test and were instructed not to intake any antibiotics, other fermented milk products, and alcoholic liquors during experimental periods. Two-hundred ml of fermented milk were given to volunteers everyday after meals for 2 weeks, and 1 week for cessation. Several kinds of selective and nonselective growth media were used for the enumeration of fecal microflora as described previously [25].

RESULTS AND DISCUSSION

Enzymatic Activities and Acid Production of *Bif. longum* MK-G7

Bifidobacteria are well known to possess some glycosidase activities and peptidases [9, 15, 18, 35, 38, 42, 43, 44]. Comparing certain enzymatic activities of bifidobacteria strains, *Bif. longum* MK-G7 showed the highest β-galactosidase and, to a lesser extent, β-glucosidase activities. On the contrary, *Bif. bifidum* Y-9 and *Bif. infantis* Y-11 showed the highest α-glucosidase and α-galactosidase activities, respectively (data not shown).

Acid and Oxygen Tolerance

Bifidobacteria ferment glucose *via* the fructose-6-phosphate shunt and this is a new pathway for the fermentation of hexoses. The Bifidus pathway yields 3 moles of acetic acid as a unique metabolite and 2 moles of lactic acid from 2 moles of glucose. Most strains of bifidobacteria, however, produce more acetic acid and less lactic acid from glucose than to be expected according to the breakdown of glucose [9, 10, 11, 44]. *Bif. longum* MK-G7 showed the highest lactic and acetic acid production among the bifidobacteria strains tested, when cultivated in MRS medium for 24 h under anaerobic cultural condition. Consequently, the ratio of lactic acid to acetic acid reached 1.22 (data not shown). Organoleptically, excessive acetic acid produced by *Bifidobacterium* gives a piquant flavor to the fermented milk. Therefore, the higher ratio of lactic acid compared with acetic acid may contribute to the more favorable flavor for the *Bif. longum* MK-G7 fermented milk. Although most strains of bifidobacteria may die rapidly unless the final pH of the product reaches above 4.6, some bifidobacteria showed various degrees of survival and viability even in lower pH values [6, 28, 33, 39]. However, the relationship and mode of action between oxygen tolerance of bifidobacteria and enzymatic activity remained to be cleared. In some lactic acid bacteria, NADH oxidase plays a major role in eliminating environmental oxygen. In bifidobacteria especially, strictly anaerobic microorganisms, NADH oxidase may play a more important role in detoxifying oxygen damage than in aerobic bacteria. Besides this, oxygen tolerance of bifidobacteria may be related to NADH peroxidase and SOD (superoxide dismutase), which are related to oxygen metabolism. *Bif. longum* MK-G7 showed a higher acid tolerance against HCl and acetic acid. Otherwise, *Bif. infantis* Y-1 showed the lowest acid tolerance and more than 4 log cycles of viable cell count decreased owing to acid injury. Additionally, the viable cell count of bifidobacteria strains tested decreased by more than 1.5 log cycles owing to oxygen toxicity, except *Bif. longum* MK-G7, *Bif. infantis* Y-2, *Bif. longum* Y-3, *Bif. longum* Y-6, and *Lb. rhamnosus* Y-7 (Table 2). The high tolerance observed for *Bif. longum* MK-G7 against acetic acid and oxygen may contribute to the high survival of this organism during the fermentation and storage periods when acidity and oxygen are the major stress factors for the *Bifidobacterium*. The strong tolerance to HCl would rather contribute to a strong survival during the stomach passage as HCl is one of the major stress factor.

Bile Tolerance and Cholesterol Assimilation

Bifidobacteria showed a great variation of bile tolerance on bile acid concentration and strain specificity [5]. Bile tolerance is known to be one of the essential properties required for bifidobacteria to survive in the small intestine and the role played in physiological functions [19, 23].

Table 2. Acid tolerance against HCl and acetic acid, and oxygen tolerance of bifidobacteria strains tested.

Test culture strains	Viability (%)			Viable cell count (cfu/ml)			
	HCl	Acetic acid	Oxygen	Control	HCl	Acetic acid	Oxygen
Bif. infantis Y-1	48.5	59.3	79.4	1.7×10^9	3.0×10^4	3.0×10^5	2.1×10^7
Bif. infantis Y-2	74.9	100.0	94.2	6.6×10^8	4.0×10^6	7.0×10^8	2.0×10^8
Bif. longum Y-3	61.7	98.5	99.0	1.4×10^9	4.5×10^5	1.1×10^9	1.2×10^9
Bif. lactis Y-4	55.2	63.4	69.1	2.1×10^9	1.4×10^5	8.0×10^5	2.7×10^6
Bif. longum Y-5	99.5	99.8	78.7	1.6×10^9	1.5×10^9	1.6×10^9	1.8×10^7
Bif. longum Y-6	86.5	100.0	96.8	7.6×10^8	4.8×10^7	8.0×10^4	4.0×10^8
Lb. rhamnosus Y-7	99.8	91.3	98.4	1.0×10^9	9.7×10^8	1.7×10^8	7.3×10^8
Bif. adolescentis Y-8	97.3	97.5	83.4	1.5×10^9	8.5×10^8	8.8×10^8	4.5×10^7
Bif. bifidum Y-9	95.7	96.4	86.2	1.9×10^9	7.5×10^8	8.9×10^8	9.9×10^7
Bif. breve Y-10	67.8	46.7	64.5	3.8×10^8	6.5×10^5	1.0×10^4	3.4×10^5
Bif. infantis Y-11	95.6	97.5	84.7	2.2×10^9	8.4×10^8	1.3×10^9	8.0×10^7
Bif. longum Y-12	99.9	100.0	81.1	3.1×10^9	3.0×10^9	3.3×10^9	5.0×10^7
Bif. longum MK-G7	99.9	100.0	88.8	1.6×10^9	1.6×10^9	1.6×10^9	1.5×10^8
Bif. longum RD-13	99.9	100.0	76.0	1.4×10^9	1.4×10^9	1.8×10^9	9.0×10^6

Table 3. Bile tolerance of bifidobacteria strains tested.

Test culture strains	Control[a]	Test[b]	Viability (%)
Bif. infantis Y-1	1.68	1.26	75.0
Bif. infantis Y-2	0.66	0.65	98.5
Bif. longum Y-3	1.43	1.51	100.0
Bif. lactis Y-4	2.07	1.50	72.5
Bif. longum Y-5	1.64	-[c]	-
Bif. longum Y-6	0.76	0.84	100.0
Lb. rhamnosus Y-7	1.00	0.10	10.0
Bif. adolescentis Y-8	1.51	1.41	93.4
Bif. bifidum Y-9	1.87	1.46	78.1
Bif. breve Y-10	0.38	0.11	28.9
Bif. infantis Y-11	2.16	-[c]	-
Bif. longum Y-12	3.06	0.17	5.6
Bif. longum MK-G7	1.60	0.93	58.1
Bif. longum RD-13	1.40	1.67	100.0

[a]Control (10^9 cfu/ml) was the number of viable cell count which grew on MTP plate medium.
[b]Test (10^9 cfu/ml) was the number of viable cell count which grew on MTP plate medium containing 0.25% (w/v) ox-bile (Oxoid).
[c]The number of viable cells was below 1.0×10^7 cfu/ml

Table 4. Cholesterol assimilation of bifidobacteria strains during anaerobic growth in MRS broth containing cholesterol (0.04%) and in 0.3% bile acid-containing growth medium.

Test culture strains	Cholesterol reduction (%)	
	Cholesterol (0.04%)[a]	Bile acid (0.3%)[b]
Bif. infantis Y-1	25.4	18.1
Bif. infantis Y-2	27.3	29.5
Bif. longum Y-3	25.2	15.1
Bif. lactis Y-4	26.5	23.0
Bif. longum Y-6	27.9	0
Lb. rhamnosus Y-7	31.3	0
Bif. adolescentis Y-8	15.8	30.8
Bif. bifidum Y-9	24.6	23.8
Bif. breve Y-10	31.4	11.0
Bif. infantis Y-11	-	14.3
Bif. longum MK-G7	28.3	49.8
Bif. longum RD-13	30.7	57.4

[a]Cholesterol (0.04%) was added to MRS broth [b]Bile acid (0.3%) was added to growth medium

Among the bifidobacteria strains tested, *Bif. infantis* Y-2, *Bif. longum* Y-3, *Bif. longum* Y-6, and *Bif. longum* RD-13 showed the highest bile tolerance; however, *Bif. longum* MK-G7 showed a medium level of bile tolerance (Table 3). Using a dynamic model of the small intestine, Marteau *et al.* [32] showed that bile exerted a strong influence on the survival of the *Bifidobacterium* and stated that the investigation of sensitivities of potential probiotic strains to bile as a selection step is very important. However, the specific mechanism for the bile resistance awaits further research. In addition, many researchers [21, 30] reported that intake of yoghurt may lower the serum cholesterol level. The cholesterol-lowering effect was known to depend largely upon species and strain specificity. *Bif. longum* MK-G7 showed a higher degree of cholesterol assimilation at the level of 28.3%, followed by *Bif. breve* ATCC 15700 and *Bif. longum* RD-13. However, *Bif. adolescentis* ATCC 15706 showed the lowest degree of cholesterol assimilation at the level of 15.8%. When 0.3% bile acid was added in the growth medium which already contained 360 μg/ml of cholesterol, *Bif. longum* MK-G7 and *Bif. longum* RD-13 showed the highest cholesterol reduction ability among the bifidobacteria strains tested (Table 4).

Antibiotic Resistances

From the viewpoint of biotherapeutic selection of bifidobacteria, investigation of the antibiotic sensitivity of bifidobacteria is regarded as a useful reference for the patients under antibiotic treatment and intake. Indeed, bifidobacteria showed different sensitivities to each antibiotic.

Table 5. Median value of the MIC (minimum inhibitory concentration) of various bifidobacteria strains against tetracycline and penicillin-G.

Test culture strains	Tetracycline (mg/l)	Penicillin-G (mg/l)
Bif. infantis Y-1	24.8<	0.52
Bif. infantis Y-2	24.8	0.52
Bif. longum Y-3	24.8	0.52<
Bif. lactis Y-4	24.8<	0.52
Bif. longum Y-5	6.2	0.52<
Bif. longum Y-6	24.8	0.52
Lb. rhamnosus Y-7	6.2	0.52
Bif. adolescentis Y-8	24.8	0.52<
Bif. bifidum Y-9	24.8	0.52<
Bif. breve Y-10	3.1	0.26
Bif. infantis Y-11	12.4	0.26
Bif. longum Y-12	<1.55	0.52<
Bif. longum MK-G7	24.8<	0.52<
Bif. longum RD-13	24.8<	0.52

Bif. longum MK-G7, *Bif. longum* RD-13, *Bif. infantis* Y-1, and *Bif. lactis* Y-4 showed much higher tetracycline resistance (>24.8 mg/l). Additionally, *Bif. longum* MK-G7, *Bif. longum* Y-3, *Bif. longum* Y-5, *Bif. adolescentis* ATCC 15706, *Bif. bifidum* ATCC 29539, and *Bif. longum* ATCC 15707 showed much higher penicillin-G resistance (>0.52 mg/l). Therefore, only *Bif. longum* MK-G7 showed the highest antibiotic resistances against both tetracycline and penicillin-G (Table 5). If antibiotic resistance trait is coded in the plasmid, the transmittance of the antibiotic resistance to other pathogenic microorganism could occur. However, we have not find any antibiotic resistance-related plasmid in *Bif. longum* MK-G7 yet.

Anti-mutagenicity and Immunopotentiating Activity

Cassand *et al.* [4] provided information on the antimutagenic properties of milk cultured with bifidobacteria. *Bif. longum* MK-G7, *Bif. lactis* Y-4, *Bif. longum* Y-6, and *Bif. bifidum* ATCC 29539 showed more than 80% of anti-mutagenicity against NQO among the bifidobacteria strains tested (Table 6). In addition to this, bifidobacteria play a significant role in resistance to infection. Many research workers [29, 35, 36, 37, 46, 47, 48] have studied the immunopotentiating activities of bifidobacteria. In order to investigate the effects of lactic acid bacteria on immunomodulating activity, Schiffrin *et al.* [45] administered bifidobacteria-containing yoghurt to volunteers and analyzed changes of blood lymphocytes and leukocyte phagocytic activity. The production of TNF (tumor necrosis factor)-α, IL (interleukin)-6, and NO (nitric oxide) increased depending on the increment of *Bif. longum* MK-G7 cell concentration (Table 7). Accordingly, it was considered that *Bif. longum* MK-G7 had immunopotentiating activity *in vitro*. Since the antimutagenicity and macrophage activating ability are strain specific, it would be of great interest to examine the cell component that plays a role in these aspects. Indeed, we observed that the cell wall component was more involved in TNF-α production and the cytoplasmic fraction in IL-6 production, respectively (data not shown). Therefore, the results suggested that the macrophage stimulatory activity was affected by the dose, strain, and composition of the bifidobacteria strains.

Table 6. Anti-mutagenicity of bifidobacteria strains against NQO (4-nitroquinoline-1-oxide).

Test culture strains	Anti-mutagenicity (%)
Bif. infantis Y-1	70.4
Bif. infantis Y-2	60.4
Bif. longum Y-3	79.0
Bif. lactis Y-4	97.6
Bif. longum Y-6	97.4
Lb. rhamnosus Y-7	11.2
Bif. adolescentis Y-8	79.7
Bif. bifidum Y-9	81.6
Bif. breve Y-10	43.6
Bif. infantis Y-11	78.0
Bif. longum MK-G7	87.3
Bif. longum RD-13	65.3

Table 7. Effect of *Bif. longum* MK-G7 on the production of cytokines such as TNF-α and IL-6, and on NO (nitric oxide) production by the Raw 264.7 macrophage cell line.

	Bifidobacteria cell concentration (μg/ml)			
	0	10	50	250
TNF-α (nM)	2.2	9.0	18.5	31.2
IL-6 (nM)	3.0	2.5	13.0	42.0
NO (μM)	42	56	69	83

Acute Toxicity and Human Clinical Test

Although acute toxicity tests were originally designed for chemicals, they also give an indication of any harmful effects associated with extremely high doses of freeze-dried bacteria [13]. When *Bif. longum* MK-G7 was administered at cell concentrations of 1, 2, 4, and 6 g/kg of body weight, all of the mice in the feeding groups survived and did not show any observable abnormalities (Table 8). Therefore, it was concluded that *Bif. longum* MK-G7 did not cause acute toxicity at all. Additionally, there was no significant changes in body weight, mobility, and

Table 8. Body weight variation of the mice before and after feeding of *Bif. longum* MK-G7 during the experimental period for all treatments.

Feeding groups	Dosage (g/kg of body weight)				
	0	1	2	4	6
Before feeding (g)	25.1	25.5	24.7	26.5	26.2
After feeding (g)	27.0	27.1	25.9	27.8	31.5

Table 9. Effect of *Bif. longum* MK-G7 administration on fecal microflora in volunteers (unit : log cfu/g feces).

Species	Samples with	Before feeding	1 week after feeding	2 weeks after feeding	1 week after cessation of feeding
Total anaerobes	MK-G7[a]	9.48	9.73	9.50	9.13
	Y-5[b]	9.08	9.60	9.40	8.88
Total aerobes	MK-G7	7.63	7.36	7.74	7.57
	Y-5	7.86	8.38	7.73	7.65
Bifidobacteria	MK-G7	9.35	9.63	9.57	9.08
	Y-5	8.96	9.39	9.42	8.65
Bacteroides	MK-G7	9.25	9.06	8.65	8.11
	Y-5	9.11	9.42	9.45	8.74
Lactobacilli	MK-G7	7.92	8.99	8.56	8.50
	Y-5	7.44	8.09	8.10	7.92
Streptococci	MK-G7	7.02	6.85	6.91	6.92
	Y-5	7.69	7.95	7.45	7.33
E. coli	MK-G7	7.53	7.19	7.23	7.26
	Y-5	7.37	7.68	7.48	7.90

[a]*Bif longum* MK-G7.
[b]*Bif. longum* Y-5.

appearances. When liver, stomach, lung, small and large intestine, spleen, and lymph node were examined, after 7 days of administration, the size of the lymph node became bigger in the feeding groups of 4 and 6 g/kg of body weight. Subsequently, this may suggest that the immune response was enhanced by *Bif. longum* MK-G7 administration. In additionto this, when *Bif. longum* MK-G7 fermented milk was administered to volunteers, the viable cell count of total bifidobacteria and anaerobes in the feces increased up to more than 0.5 log cycles, compared with the pre-administration period. In particular, even though *Bif. longum* Y-5 did not inhibit the growth of *Bacteroides, Bif. longum* MK-G7 inhibited *Bacteroides* at the level of 1.0-1.5 log cycles (Table 9). Conclusively, our results suggest that *Bif. longum* MK-G7 may be employed as a probiotic strain for the production of fermented milk products.

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