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Immunomodulatory and antitumor effects *in vivo* by the cytoplasmic fraction of *Lactobacillus casei* and *Bifidobacterium longum*

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The immunomodulatory and antitumor effects of lactic acid bacteria (LABs) were investigated. Cytoplasmic fraction of *Lactobacillus acidophilus, Lactobacillus casei* and *Bifidobacterium longum* were tested for the antiproliferative activity *in vitro* to SNUC2A, SNU1, NIH/3T3 and Jurkat cell lines by crystal violet assay. All cytoplasmic fraction suppressed proliferation of tumor cells, though *L. casei* and *B. longum* were more effective. From these results, cytoplasmic fraction of *L. casei* and *B. longum* with Y400 as a control were administered as dietary supplements to Balb/c mice for 2, and 4 consecutive wks. Administration for 4 wks enhanced the number of total T cells, NK cells and MHC class II^+ cells, and $CD4^-CD8^+$ T cells in flow cytometry analysis. To determine of antitumor activity of LABs preparation *in vivo*, F9 teratocarcinoma cells were inoculated on mice at 14th day. Body weight was decreased with increased survival rate in all groups with the cytoplasm of LABs. Our results showed that cytoplasmic fraction of LABs had direct antiproliferative effects on tumor cell lines in vitro, effects on immune cells *in vivo*, and antitumor effects on tumor-bearing mice with prolonged survival periods.

Key words: *Lactobacillus*, *Bifidobacterium*, immunophenotyping, *in vivo*, survival rate

Introduction

The enhancement of the gut mucosal barrier may prevent the invasion of pathogens and assist in handling antigens. Lactic acid bacteria (LABs), a gram-positive and non pathogenic organism, produce of lactic acid [1]. Many studies have shown the beneficial therapeutic effects of probiotic LABs. They can prevent or ameliorate diarrhea through their effects on the immune system. Moreover, They may protect infection because they compete with pathogenic viruses or bacteria for binding sites on epithelial cells [2,3,4,5] and induce systemic immune response including secretion of cytokines by directly action to mucosal lymphocytes [6,7].

Among its many therapeutic attributes, LABs have antitumor activity and inhibit metastasis [8,9,10]. LABs such as *Lactobacillus acidophilus* [9,11], *L. casei* [12,13], and *Bifidobacterium longum* [14,15,16] inhibit the growth of both implantable and chemically induced tumor cells in rodents. *Lactobacillus* has mitogenic activity, adjuvanticity and shows activating macrophages *in vivo* including cytostatic activity [17-22]. Perdigon *et al*. [33] reported that enhanced macrophage and lymphocyte activity in mice after oral administration of *L. acidophilus* and *L. casei*. Increased NK cell activity is known in mice injected with *L. casei* [19], *L. rhamnosus* [23], and yogurt containing live LAB [24].

Furthermore, whole cells, heat-killed cells, cell wall, and cytoplasmic fractions of LABs can show various functions in many works. However, most reports on antitumor activity and immunomodulatory effects of LABs, have been focused on whole cells or its membrane component, peptidoglycans, though the effect of soluble materials in food applications can be different from that of insoluble ones. As little attention has been paid for the soluble fractions, the importance of cytoplasmic fraction of LABs *in vivo* has been overlooked.

In this study cytoplasmic fraction of *L. acidophilus*, *L. casei* and *B. longum* were compared with their antiproliferative activity to tumor cells *in vitro*. From this preliminary results, the cytoplasmic fraction of *L. casei* and *B. longum*, and Y400 were chosen for further study of immunomodulation and antitumor activity in tumor-bearing mice with long-term feeding.

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Table 1. List of lactic acid bacteria for the in vitro cytotoxicity assay

Strain	Time for culturea	Final cell numberb
Lactobacillus acidophilus SNUL	11 hr	2.0×10^9
Lactobacillus casei YIT9029	18 hr	1.1×10^9
Bifidobaterium longum HY8001	18 hr with 0.05 % cystein	8.0×10^9

[a]In MRS broth at 37°C in anaerobic condition
[b]Cells per milliliter

Materials and Methods

Experimental animals

Male Balb/c mice, 6 wks old, purchased from Seoul National University, were housed in plastic cages in an air conditioned room (22 ± 2°C, humidity 55 ± 10%), and given food and water freely.

Preparation of LABs

L. acidophilus SNUL, *L. casei* YIT9029, and *B. longum* HY8001 were obtained from Hankuk Yakult Institute (Yongin, Korea). Anaerobic culture condition in anaerobic jar (BBL) with catalysts (DIFCO) was described in Table 1. After cultivation, the cells were harvested by centrifugation and washed and resuspended in distilled water for disruption with French Press at 2,000 $g \cdot s \cdot cm^{-2}$. Cytoplasmic fractions were the supernatant of ultracentrifugation at $70000 \times g$ for 30 min. Samples were sterilized with a 0.2-μm filter and kept at –80°C.

Tumor cells

SNU1 (human gastric cancer cells), SNUC2A (human colorectal carcinoma cells), NIH/3T3 (mouse embryo fibroblast cells), F9 (teratocarcinoma cells), and Jurkat (human acute T cell leukemia cells) were obtained from KCLB (Korean Cell Line Bank, Seoul, Korea) and maintained in RPMI medium containing 10% (v/v) fetal bovine serum (FBS, GibcoBRL, Grand Island, NY) in a humidified atmosphere with 5% CO_2 at 37°C.

Measurement of antiproliferative activity *in vitro*

To evaluate the antiproliferatie activity of samples, cytotoxicity assay was performed with crystal violet dye for the quantitative analysis of cell numbers as a total protein amount. Cells were plated at a density of 5×10^4 cells per well for NIH/3T3, SNU1, and SNUC2A, and of 10^4 cells per well for Jurkat cells in 96-well plates. Six hrs later, serial dilution of cytoplasmic fraction of LABs from 250 μg/ml was added to wells, and incubated for 72 hrs. Washing with phosphate-buffered saline (PBS, pH 7.2), plates were fixed with 1% glutaraldehyde (Wako), and stained with 0.2% crystal violet solution (Merck). Five min later, wells were washed with tap water and 1% SDS (Sigma) was added. Absorbance was measured by ELISA plate reader (BIO-RAD model 550) at 540 nm for the cytotoxicity calculation.

$$\% \text{ Cytotoxicity} = \frac{\text{Control O.D} - \text{Sample O.D}}{\text{Control O.D}} \times 100$$

Experimental design for *in vivo* study

The mice, assigned to 14 groups (Table 2), were fed with cytoplasmic fraction of *L. casei* (100 mg/kg/day), B. longum (100 mg/kg/day), and whole cells of Y400 (2.6 ml/kg/day, HanKuk Yakurt Institute) as the control sample. Distilled water (D.W.) was used to substitute LABs in adjusting feeding condition of experimental groups. To evaluate the antitumor effects, F9 teratocarcinoma cells (1×10^6 cells/mouse) were inoculated *i.p.* at the day of 14th. The change of body weights was measured at the intervals of 5 days, and survival rate was assessed. Statistics were done with Student's t-test.

Immunophenotyping by flow cytometry analysis

Blood was collected from tail vein of mice. The PBMCs were obtained from each blood sample with 0.5 μM EDTA (Sigma) in PBS by centrifugation. The red blood cells were removed by BCL buffer. A total of 1×10^6 cells were incubated with each of FITC-conjugated rat anti-mouse CD3, mouse anti-mouse Ly-49A^{B60} (PharMingen, BD Biosciences), mouse anti-mouse MHC class II I-A^b/I-A^d (Serotec, Inc., Raleigh, NC) for 30 min at 4°C. For two-color flow cytometry analysis, a rat anti-mouse CD4-CyChrome and a rat anti-mouse CD8-RPE (Serotec) were used. Flow cytometry analysis was performed in a FACSCalibur with CellQuest program (Becton Dickinson).

Table 2. Summary of experimental groups

Group[a]	Treatment[b]
Control	D.W. for 4 wks
B2	*Bifidobacterium longum* for 2 wks and D.W. 2 wks
B4	*Bifidobacterium longum* for 4 wks
L2	*Lactobacillus casei* for 2 wks and D.W. 2 wks
L4	*Lactobacillus casei* for 4 wks
Y2	Y400 for 2 wks and D.W. 2 wks
Y4	Y400 for 4 wks

[a]Five mice in each group.
[b]Oral administration of cytoplasmic fraction of *B. longum* (B) and *L. casei* (L) at 100 mg/kg/day, and Y400 (Y) at 2.6 ml/kg/day as a dietary supplement.

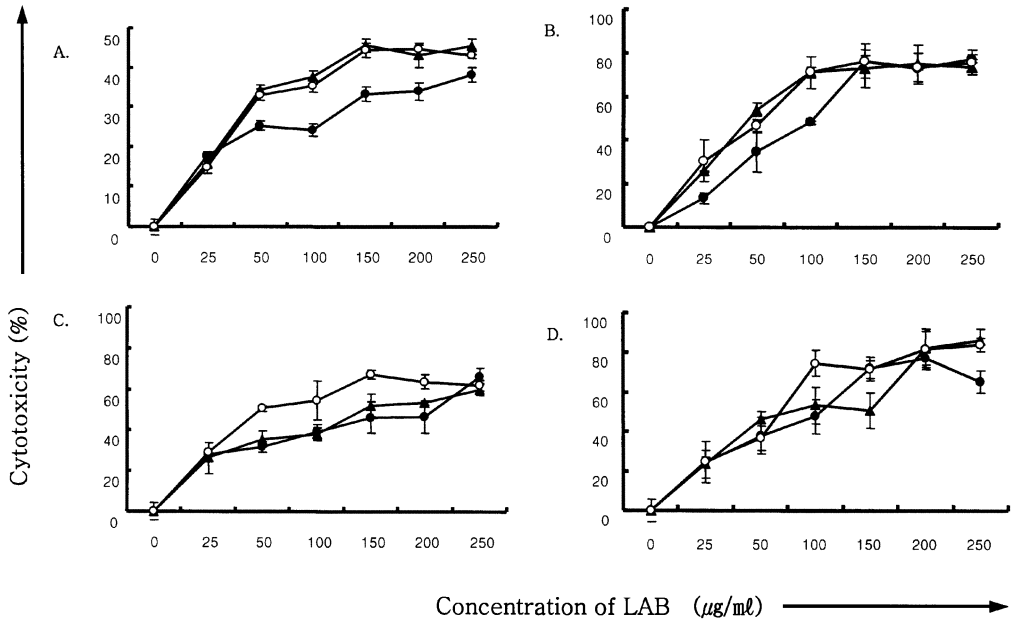

Fig. 1. Cytotoxicity of LABs on tumor cell lines. A; SNUC2A, B; NIH/3T3, C; SNU1, and D; Jurkat. Cytoplasmic fraction of *L. acidophilus* (●), *L. casei* (○) and *B. longum* (▲) were added to tumor cells at 6 hrs of incubation for 72 hrs incubation. Proliferation of cells was quantified as % cytotoxicity by crystal violet assay.

Table 3. Antiproliferative effect of LABs in cytotoxicity assay

Cytoplasmic fraction[a]	Tumor cell lines			
	SNUC2A	NIH/3T3	SNU1	Jurkat
L. acidophilus	24.5 ± 2.4[b]	35.1 ± 5.7	47.3 ± 2.3	34.9 ± 7.6
L. casei	36.1 ± 2.4	76.5 ± 3.7	60.9 ± 5.6	36.8 ± 7.6
B. longum	36.0 ± 3.0	67.5 ± 4.8	43.1 ± 8.7	44.3 ± 4.5

[a]Concentration at 50 μg/ml was used.
[b]Mean (%) ± S.D. from three independent cultures. Each experiment was done in triplicate.

Results

Antiproliferative activity of cytoplasmic fraction of LABs *in vitro*

All cytoplasmic fraction of LABs showed strong antiproliferative effect to tumor cells (Fig. 1). In particular, the cytoplasmic fractions of *L. casei* and *B. longum* were more effective with inhibition rates around 50% at 50 μg/ml (Table 3). These strains were selected for further study *in vivo*.

Increased $CD3^+$ cells, NK cells and MHC class II^+ cells in PBMCs

To access the effect of cytoplasmic fraction of LABs on cellular immunity, preparation of LABs was administered as a dietary supplement for 2 and 4 wks. Four wks later, increased $CD3^+$ T cells were observed in all groups. Longer intake of LABs showed more effects as the all of 4 wks groups showed above increase of 70 % (Fig. 2). NK cells and MHC class II^+ cells which are antigen presenting cells like dendritic cells, activated macrophages and some of B cells also showed similar increment (Fig. 3 and 4).

Increased $CD8^+$ T cells in two color analysis of flow cytometry

The change of T cell subsets was observed after oral administration of cytoplasmic fraction of LABs. Four wks later, we found the proportion of $CD4^-CD8^+$ T cells and double positive T cells were increased (Fig. 5). Interestingly, no changes was observed in $CD4^+CD8^-$ T cells, resulting decreased $CD4^+/CD8^+$ ratio. The summary of numbers was shown in Table 4.

Change of body weight in F9-bearing mice

During administration of cytoplasmic fraction of LABs for 4 wks, F9 was inoculated into mice at 2 wks. Though the body weight of mice before tumor inoculation were same in all groups, it was decreased by time in tumor-bearing groups with the treatment of cytoplasmic fraction of LABs. There was no difference between 2 and 4 wks feeding groups (Fig. 6).

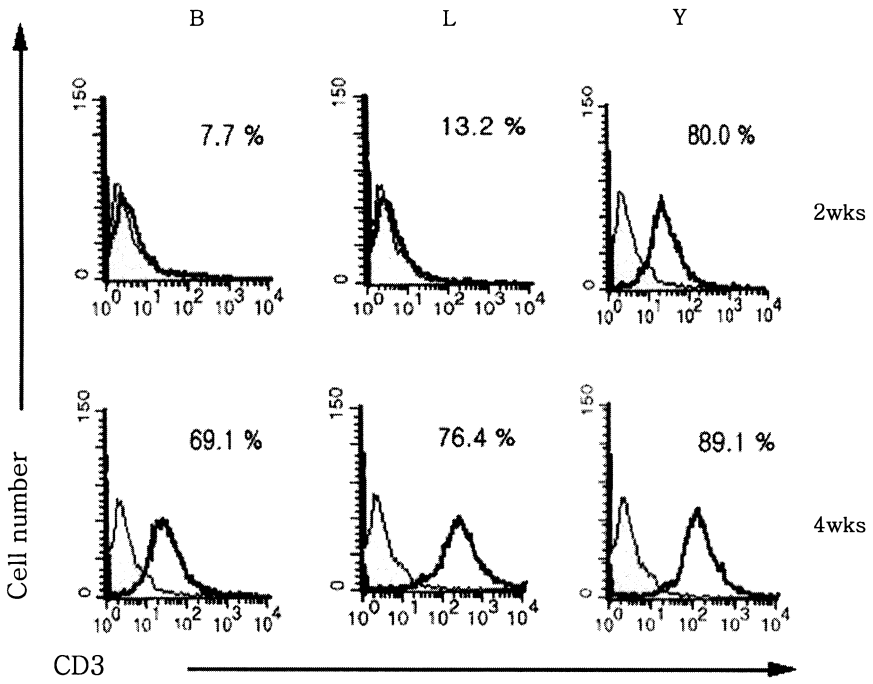

Fig. 2. Change of $CD3^+$ T cells after administration of LABs. The mice were orally administered the cytoplasmic fraction of *B. longum* (B) and *L. casei* (L), and Y400 (Y) for 2 or 4 wks. PBMCs were analyzed for the cell surface CD3 expression by flow cytometry. The percentage of increase compared to control which was not given LABs was shown.

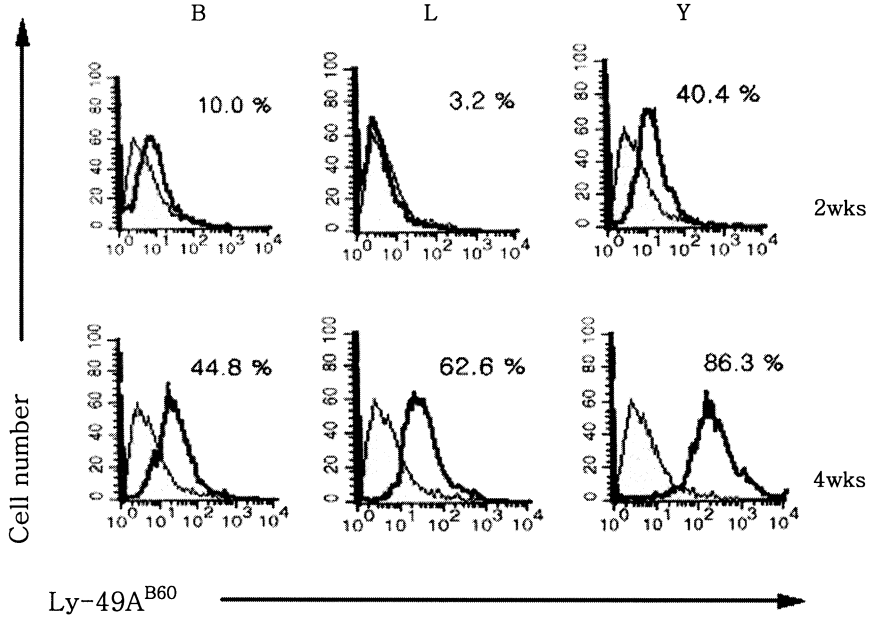

Fig. 3. Change of NK cells after administration of LABs. The mice were orally administered cytoplasmic fraction of *B. longum* (B) and *L. casei* (L), and Y400 (Y) for 2 or 4 wks. PBMCs of mice were analyzed for Ly-49A^{B60}, a cell surface marker for NK cells, by flow cytometry. The percentage of increase compared to control which was not given LABs was shown.

Survival rate in F9-bearing mice

The mice in control group began to die from 26th days after inoculation of F9 cells and died all on 44th days. Significant prolonged survival was observed in LABs treated groups. Mean survival rate of *B. longum* group for 4 wks, and Y400 for both 2 wks and 4 wks were remarkably increased as shown to be 80%, 60% and 80% on 44th days (Fig. 7).

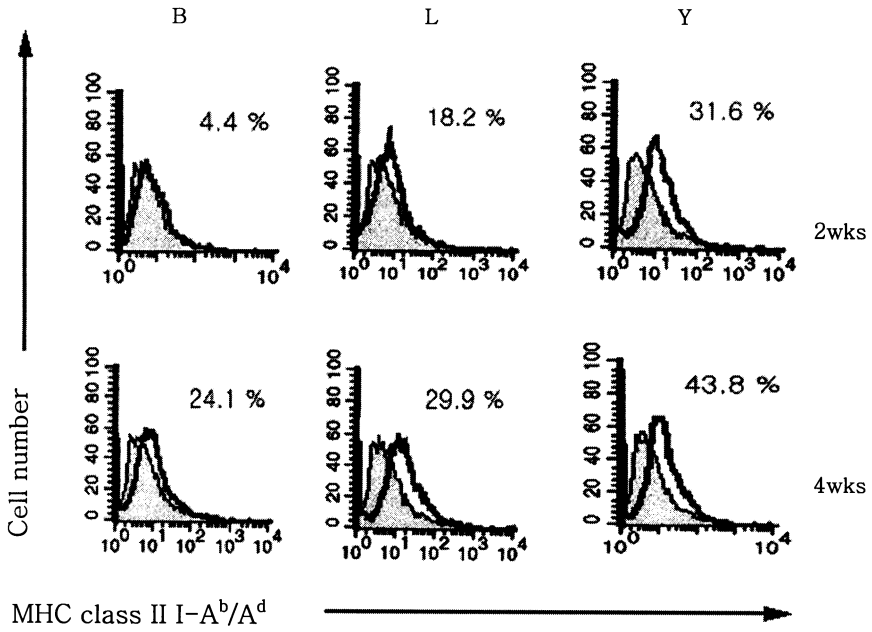

Fig. 4. Expansion of MHC class II$^+$ cells after administration of LABs. The mice were orally administered cytoplasmic fraction of *B. longum* (B) and *L. casei* (L), and Y400 (Y) for 2 or 4 wks. PBMCs of mice were analyzed for the cell surface MHC class II expression by flow cytometry. The percentage of increase compared to control that was not given LABs was shown.

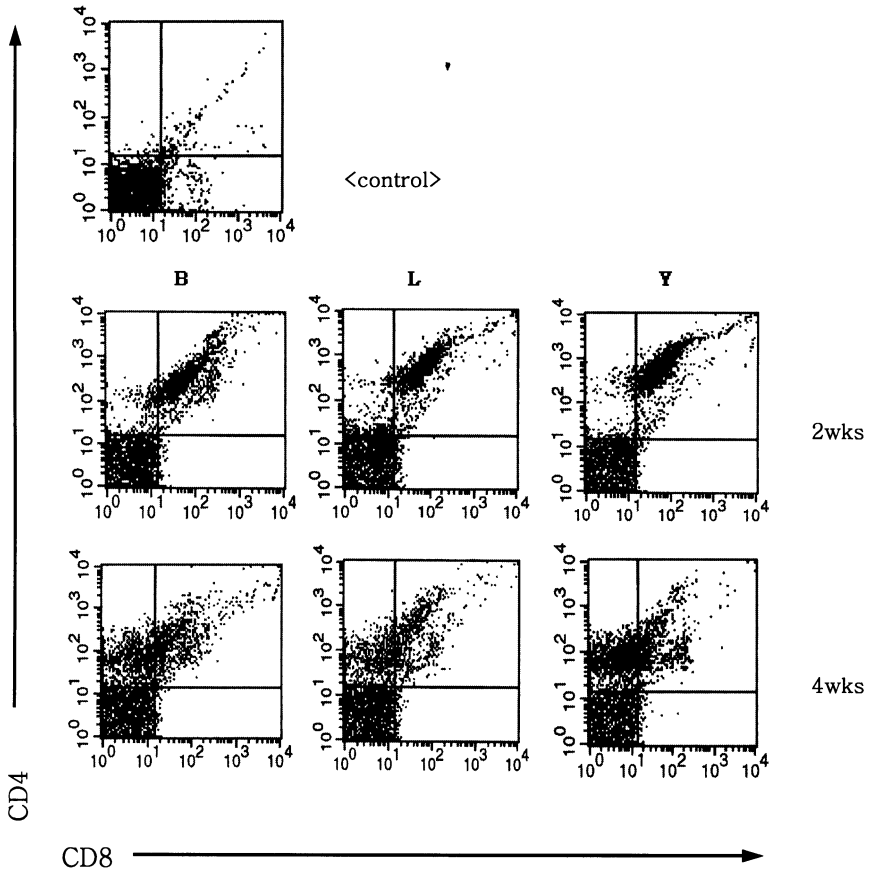

Fig. 5. Double staining of PBMC for CD4 and CD8 after administration of LABs. The cells were taken from mice that were given cytoplasmic fraction of *B. longum* (B) and *L. casei* (L), and Y400 (Y) as dietary supplement for 2 or 4 wks. Control was PBMC from a group that was not given LABs.

Table 4. Change of T cell subsets with feeding of LABs

Group	% of phenotypes[a]			
	$CD4^-CD8^-$	$CD4^+CD8^-$	$CD4^-CD8^+$	$CD4^+CD8^+$
Control	65.3 ± 1.7[b]	15.5 ± 0.4	1.7 ± 0.2	17.6 ± 2.4
B2	24.5 ± 10.1*	12.0 ± 0.9	7.0 ± 0.2*	56.5 ± 9.5*
B4	29.0 ± 5.1*	12.3 ± 0.7	6.9 ± 1.7*	51.8 ± 3.0*
L2	35.7 ± 10.5*	10.2 ± 0.0	10.8 ± 5.9*	43.3 ± 9.3*
L4	22.4 ± 8.5*	11.0 ± 0.8	12.0 ± 0.8*	54.6 ± 8.6*
Y2	16.3 ± 2.0*	11.3 ± 0.5	17.1 ± 3.9*	55.4 ± 1.6*
Y4	10.1 ± 4.9*	11.3 ± 0.3	25.3 ± 7.1*	53.2 ± 2.9*

[a]Double stained PBMC for CD4 and CD8 were analyzed by flow cytometry with Cell Quest program.
[b]Mean ± S.D. (n=5, *P ≤ 0.05)

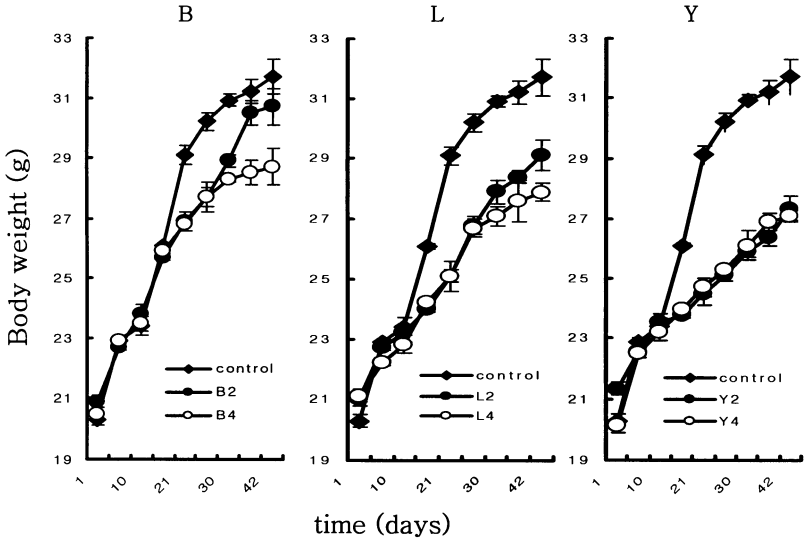

Fig. 6. Effect of LABs on body weight change in F9-bearing mice. Cytoplasmic fraction of *B. longum* (B) and *L. casei* (L), and Y400 (Y) were administrated as dietary supplement for 2 or 4 wks. F9 cells were inoculated on day 14. Control was not given LABs. The values are expressed as the mean ± SD (n = 5).

Discussion

To examine direct antiproliferative effect of cytoplasmic fraction of *L. acidophilus*, *L. casei* and *B. longum*, we conducted cytotoxicity assay on colon cancer, gastric cancer, and acute T cell leukemia cells with NIH/3T3, a fibroblast cell line used on general cytotoxicity assay. The cytoplasmic fractions of LABs were found to have anti-proliferative effect *in vitro* on tumor cells. In particular, the cell fraction of both *L. casei* and *B. longum* showed high activity on all tumor cells, and led us in vivo antitumor study with these strains. Our data showing different antiproliferative activity in these strains consist with the result of Pessi *et al.* [25].

Despite of an immune change with the challenge can be measured more than 2 wks, most of previous *in vivo* studies was done for one week or less [11]. Feeding mice with LABs in long period is necessary for the evaluation of cellular immunity by probiotics. By feeding of LABs before and after F9 tumor inoculation, the body weight was decreased compared to control suggesting tumor growth was controlled and restrained by potentiated host immunity. This observation was sustained by the increased survival rate in 4-wks feeding group than 2-wks. Similar results was reported by other researchers with the direct intraperitoneal injection of *L. casei* 9018 against the sarcoma-180 [19,27].

For the antitumor activity of LABs *in vivo*, the increased specific tumor immunity in probiotic treated mice was from activated immune cells, not by direct killing on tumor cells, in the study with *Streptococcus thermophilus* on chemically induced tumor [28]. In our experiment, increased $CD8^+$ T cell subset was observed in long-term feeding groups with a profound change of other immune cells, indicating feeding of preparation of LABs modify cellular immunity. The increased $CD4^+/CD8^+$ T cells may suggest the stage of body against immune stimulation before maturation as the single positive T cells, though its exact nature is not clear. As

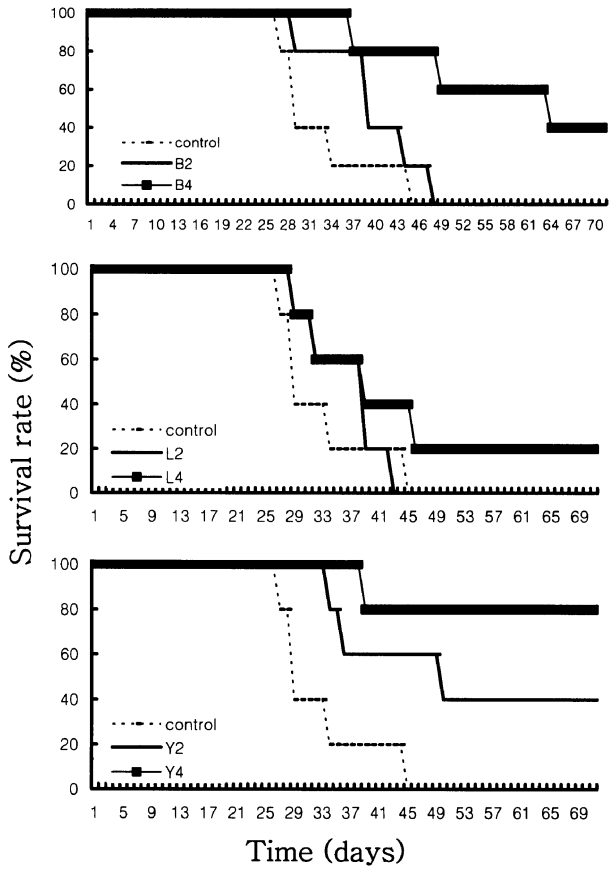

Fig. 7. Survival rate of F9-bearing mice after LABs administration. Cytoplasmic fraction of *B. longum* (B) and *L. casei* (L), and Y400 (Y) were administrated as dietary supplement for 2 or 4 wks. F9 cells were inoculated on day 14. Control was not given LABs.

already known, the final effector cells in tumor immunity are $CD8^+$ cytolytic T lymphocytes, MHC class II^+ cells like activated macrophages and dendritic cells, and NK cells [29-32]. The $CD8^+$ T cells increased while $CD4^+$ T cells were in marginal change in our experiments. This observation is consist with the report with the intraperitoneal injection of *L. casei* [28]. Furthermore, MHC class II^+ cells and NK cells were increased with long-term feeding of LABs, and this can be one of factors for the improved antitumor immunity in this study.

Though the mechanism on different degree of antitumor activity against F9 cells and on effects to immune cell populations by strains of LABs is not clear, considering different survival rate in groups, our results are consist with the report on proliferation of hepatoma cells in *L. casei* and *B. longum* treated group [32]. The different survival rates in Y400, *L. casei*, and *B. longum* fed groups may also reflect the difference of sample preparation as whole cell body and cytoplasmic fraction, though it is not likely because same cytoplasmic preparation from *L. casei*, and *B. longum* showed different survival rate in mice.

In this study, oral administration of cytoplasmic preparation of LABs as a dietary supplement is found to have antitumor effects *in vivo* with the modulation of celluar immunity, suggesting that both *L. casei* and *B. longum* in intestinal microflora can activate immune system to prevent diseases including tumors.

References

1. **Bloksma N, de Heer E, van Dijk H, Willers JM.** Adjuvanticity of *lactobacilli*. I. Differential effects of viable and killed bacteria. Clin Exp Immunol. 1979, **37**, 367-375.
2. **Bogdanov IG, Popkhristoveand P, Marinov L.** Anti cancer effect of *Antibioticum bulgaricum* on Sarcoma-180 and the solid form of Ehlich carcinoma. Abstr VIII Intl Cancer Congress, p. 354-365, Moscow, 1962.
3. **De Simmone C, Bianchi Salvadori B, Negri R.** The adjuvant effect of yogurt on production of gamma-interferon by ConA-stimulated human peripheral blood lymphocyte. Nutr Rep Int 1986, **33**, 419-433.
4. **Erickson KL, Hubbard NE.** Probiotic immunomodulation on health and disease. J Nutr 2000, **130**, 403-409.
5. **Gill HS.** Stimulation of the immune system by lactic culture. Int Dairy J 1998, **8**, 535-544.
6. **Gill HS, Rutherfurd KJ, Prasad J, Gopal PK.** Enhancement of natural and acquired immunity by *Lactobaillus rhamnosus*, *Lactobacillus acidophilus* and *Bifidobacterium lactis*. Br J Nutr 2000, **83**, 167-176
7. **Goldin BR, SL Gorbach.** Effect of *Lactibacillus acidophilus* dietary supplements on 1,2-dimethylhydrazine dehydrochloride-induced intestinal cancer in rats. J Natl Cancer Congress Inst 1980, **64**, 263-265.
8. **Hanna N, Fidler IJ.** Role of natural killer cells in the destruction of circulating tumor emboli. J Natl Cancer Inst 1980, **65**, 801-809
9. **Hashimoto S, Nomoto K, Matsuzaki T, Yokokura T, Mutai M.** Oxygen radical production by peritoneal macrophages and Kuffer cells elicited with *Lactobacillus casei.* Infect Immun 1984, **44**, 61-67.
10. **Hashimoto S, Nomoto K, Nakaoka M, Yokokawa T.** *In vitro* and *in vivo* release of cytostatic factors from *Lactobacillus casei*-elicited peritoneal macrophages after stimulation with tumor cells and immunostimulants. Cancer Immunol Immunother 1987, **24**, 1-7.
11. **Havenaar R, Huis, in't Veld.** Probiotics: a general view. In The Lactic Acid Bacteria in Health and Disease, pp. 209-224, 1992.
12. **Hove H, Norgaard H, Mortensen BP.** Lactic acid bacteria and human gastrointestinal tract. Eu J Clin Nutr 1999, **53**, 339-50.
13. **Kaila K, James C.** Survival and therapeutic potential of probiotic organism with reference to *Lactobacillus acidophilus* and *Bifidobacterium* spp. Immuno Cell Biol 2000, **78**, 80-88.
14. **Kato I., Endo K, Yokokura T.** Effects of oral administration of *Lactobacillus casei* on antitumor response induced by tumor resection in mice. Int J Immunopharmacol 1994, **16**,

29-36.
15. **Kato I, Kobayashi S, Yokokura T, Mutai M.** Antitumor activity of *Lactobacilluse casei* in mice. Gann 1981, **72**, 512-23.
16. **Kato I, Kobayashi S, Yokokura T.** Lactic acid bacterium potently induced the production of interlukin-12 and interferon-gamma by mouse splenocyte. Int J Immunopharmacol 1999, **21**, 121-131.
17. **Kato I, Yokokura T, Mutai M.** Macrophage activation by *Lactobacillus casei* in mice. Microbiol Immunol 1983, **27**, 611-18.
18. **Kato I, Yokokura T, Mutai M.** Augmentation of mouse natural killer cell activity by *Lactobacillus casei* and its surface antigens. Microbiol Immunol 1984, **28**, 209-217.
19. **Kelkar SM, Shenoy MA, Koklij GS.** Antitumor activity of lactic acid bacteria on a solid fibrosarcoma, sarcoma-180 and Ehrlich ascites carcinoma. Cancer Lett 1988, **42**, 73-77.
20. **Kim J, Woo HJ., Kim YS., Kim KH, Lee HJ.** Cell cycle dysregulation induced by cytoplasmic of Lactococcus latis ssp. lactis in SNUC2A, a colon cancer cell line. Nutr Cancer 2003, **46**, 197-201
21. **Kulkami N, Reddy BS.** Inhibitory effect of *Bifidobacterium longum* cultures on the azyoxymethane-induced aberrent crypt foci formation and fecal bacterial β-glucuronidase. Proc Soc Exp Biol Med 1994, **207**, 278-283.
22. **Matsuzaki T, Yokokura T, Mutai M.** Antitumor effect of intrapleural administration of *Lactobacillus casei* in mice. Caner Immunol Immunother 1988, **26**, 209-214.
23. **Matsuzaki T.** Immunomodulation by treatment with *Lactobacillus casei* strain shirota. Int J Food Microbiol 1998, **41**, 133-140.
24. **McIntosh GH, Royle PJ, Playne MJ.** A probiotic strain of *L. acidophilus* reduces DMH-induced large intestinal tumors in male Sprague-Dawley rats. Nutr Cancer 1999, **35**, 153-159.
25. **Mugitani H, H Furue.** Evaluation on the safety of LC9018-study of single subcutaneous administration of LC 9018 to healthy men. Biotherapy 1987, **1**, 286-289.
26. **Perdigon G, de Macias ME, Alvarez S, Oliver G, de Ruiz Holgado AA.** Effect of perorally administered *lactobacilli* on macrophage activation in mice. Infect Immun 1986, **53**, 404-410.
27. **Pessi T, Sutas Y, Saxelin M, Kallioinen H, Isolauri E.** Antiproliferative effects of homogenates derived from five strains of Candidated probiotic bacteria. Appl Environ Microbiol 1999, **65**, 4725-4728.
28. **Pool-Zobel BL, Neudecker C, Domizlaff I, Ji S, Schillinger U, Rumney C, Moretti M, Vilarini I, Scassellati-Sforzolini R, Rowland I.** *Lactobacillus*- and *Bifidobacterium* -mediatd antigenotoxicity in the colon rats. Nutr Caner 1996, **26**, 365-380.
29. **Singh J, Rivenson A, Tomita M, Shimamura S, Ishibashi N, Reddy BS.** *Bifidobacterium longum*, a lactic acid-producing intestinal baterium, inhibits colon cancer and modulates the intestinal biomarkers of colon carcinogenesis. Carcinogenesis 1997, **18**, 833-841.
30. **Skakelford LA, Rao DR, Chawan CB, Pulusani SR.** Effect of feeding fermented milk on the chemically induced colon tumors in rats. Nutr Cancer 1983, **5**, 159-164.
31. **Talmadge JE, Meyers KM, Prieur DJ, Starkey JR.** Role of natural killer cells in tumor growth and metastasis: C57BL/6 normal and beige mice. J Natl Cancer Inst 1980, **65**, 929-935.
32. **Tomita K, Akaza H, Nomoto K, Yokokura T, Matsushima H, Homma Y, Aso Y.** Influence of *Lactobacillus casei* on rat bladder carcinogenesis. Nippon Hinyokika Gakkai Zasshi 1994, **85**, 655-663.
33. **Yokokura T.** Antitumor and immunostimulating activities of orally administered *Latobacillus casei.* In The 3rd International Symposium on intestinal Flora. pp. 72-88, Intestinal Flora and Human Health, 1994.