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REVIEW
Lactococcus sp. as Potential Probiotic Lactic Acid Bacteria

Hiromi KIMOTO-NIRA*, Koko MIZUMACHI, Masaru NOMURA, Miho KOBAYASHI, the late Yasuhito FUJITA, Takashi OKAMOTO[1], the late Ichirou SUZUKI, Noriko M. TSUJI[2], Jun-ichi KURISAKI[3] and Sadahiro OHMOMO

National Institute of Livestock and Grassland Science
(Tsukuba Norin-danchi, P.O. Box 5, Ibaraki 305–0901, Japan)

Abstract
The most widely used probiotic bacteria are lactobacilli and bifidobacteria, which have been isolated from the human gastrointestinal tract. The development of new probiotic strains, which are more feasible and beneficial organisms, is awaited in the dairy industry. Lactococci would be promising species because of their extensive usage in manufacturing dairy products such as cheese and fermented milk. However, there have been few studies on the probiotic activity of lactococci since they are traditionally not considered to be natural inhabitants of the human gastrointestinal tract. Recently, several works showed the possibility of the presence of lactococci in the flora of the human or animal gastrointestinal tract. In this review, we would like to propose *Lactococcus* sp. as new probiotic bacteria.

Discipline: Food
Additional key words: Caco-2, Cholesterol-removing ability, Immunomodulatory activity, probiotics

Introduction

In recent years, probiotic activity of lactic acid bacteria has been emphasized. Originally Fuller[5] defined probiotics as live microbial feed supplements which beneficially affect the host animal by improving its intestinal microbial balance. Lately, probiotics are commonly recognized as viable microorganisms that exhibit a beneficial effect on the health of the host when they are ingested[26]. For example, the ability to reduce serum cholesterol levels, productivity of antimicrobial substrates, immunomodulatory activity, etc. are considered as effective properties of probiotics.

The most widely used probiotic bacteria are lactobacilli, a genus of lactic acid bacteria, and bifidobacteria. One of the reasons is that the two species are commonly isolated lactic acid bacteria from the intestine, so they can survive and function in the intestine. Another reason is that they do not have any harmful effects on the host, in contrast to other intestinal bacteria. The development of new probiotic strains, which are more feasible and beneficial organisms, is awaited in the dairy industry. Lactic acid bacteria consist of twenty genera. Among them, lactococci play an essential role in manufacturing fermented milk products. They can grow well in milk while some of the probiotic cultures, such as *Lactobacillus (Lb.) acidophilus* strains, do not grow during production of fermented milk products. So, in manufacturing fermented milk with *Lb. acidophilus* some additives, such as compounds which are resolved by protease, are used or dairy lactic acid bacteria are added as an accelerator. In contrast, these operations are not needed with lactococci. In the selection of new probiotic microorganisms, the safety of probiotic microorganisms has been of prime importance[39]. Lactococci as well as lactobacilli are most commonly given the generally-recognized-as-safe (GRAS) status. Nevertheless, there have been few studies on lac-

Present addresses
[1] National Agricultural Research Center for Hokkaido Region (Sapporo, Hokkaido 062–8555, Japan)
[2] National Institute of Advanced Industrial Science and Technology (Tsukuba, Ibaraki 305–8566, Japan)
[3] Jumonji University (Niiza, Saitama 352–8510, Japan)
*Corresponding author: e-mail anne@affrc.go.jp
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tococci as probiotic bacteria, because the strains were formally not considered to be natural inhabitants in the intestine[44]. Once the potential of new probiotic strains is confirmed, the development of milk products made with such lactococci in addition to lactobacilli and bifidobacteria should result in new types of probiotic preparations. In this review, we would like to emphasize the probiotic properties of lactococci, such as the abilities to survive in the intestine, lower the cholesterol levels and modulate the host's immunity.

Probiotic properties

1. Survival in the intestine

One of the fundamental properties of a probiotic bacterium is its ability to survive in a sustained transient state in the gastrointestinal tract. It has been suggested that bacteria ingested as probiotics cannot affect the intestinal environment unless their population reaches a certain minimum level of between 10^6 and 10^8 cfu/g in intestinal content[31]. It has also been suggested that to give any therapeutic effect, the minimal number of viable cells of *Lb. acidophilus* and *Bifdobacterium* spp. in a product should be greater than 10^5/g [13]. In order to accumulate in the intestinal tract, the bacteria must survive at first. The ingested bacteria must be resistant to the enzymes in the oral cavity (e.g., lysozyme), as well as to the environment during the digestion process in the stomach (e.g., exposure to low pH) and the intestine (e.g., exposure to bile). There are many studies on bile and acid tolerance of lactobacilli in order to select successful probiotics, but few reports on lactococci.

The bile tolerance of seventy-three strains of lactococci for selection as probiotics was examined (Table 1). Surprisingly, some lactococci had a good ability to tolerate bile. Most *Lactococcus (Lc.) lactis* subsp. *lactis* and subsp. *lactis* bv. *diacetylactis* strains tested could grow in bile-containing broth. On the other hand, the growth of twenty-four *Lc. lactis* subsp. *cremoris* strains tested was completely inhibited by bile. It was also reported that *Lc. lactis* subsp. *lactis* is capable of displaying adaptive response to the stress by media containing 0.3% bile salts, while in *Lc. lactis* subsp. *cremoris* the response is much weaker[14]. Among the tested strains, *Lc. lactis* subsp. *lactis* bv. *diacetylactis* N7 was selected for further study because it had the best tolerance to bile of the strains tested.

Most microorganisms are destroyed by low pH and the hydrochloric acid in the stomach. In humans, the time from entrance to release from the stomach was reported to be 90 min[2] and the bactericidal effect of acid is evident at pH values below 2.5[29]. When the initial cell count of strain N7 was 9.2 log cfu/mL, it had a moderate survival rate (5.4 log cfu/mL) for 2 h at pH 2.0 or a better rate (7.8 log cfu/mL) for 2 h at pH 3.0[16]. According to the results, strain N7 was tolerant to low pH and bile, indicating that strain N7 may reach the intestine alive after ingestion.

It has been generally assumed that lactococci do not

Table 1. Growth characteristics of lactococci in various media containing bile

	Medium					
	MRS		GM17		M17	
Strains	Control	Bile	Control	Bile	Control	Bile
Lactococcus lactis subsp. *lactis*						
ATCC 19435	1.10	–	1.78	–	1.10	–
712	1.30	0.12	2.21	–	1.80	–
527	1.58	0.47	2.25	0.32	1.21	0.32
Lactococcus lactis subsp. *cremoris*						
ML	0.70	–	0.66	–	1.00	–
HP	0.72	–	1.77	–	0.58	–
H61	0.68	–	0.46	–	0.78	–
Lactococcus lactis subsp. *lactis* biovar *diacetylactis*						
DRC1	1.55	0.78	1.81	–	1.08	–
DRC2	1.21	0.65	1.58	0.14	1.10	0.17
N7	1.48	0.90	1.75	1.22	1.42	1.33
B75-1 W	1.52	0.28	2.12	0.09	0.91	0.17
B75-2 Y	1.49	0.31	2.12	0.04	1.38	0.11

Each MRS, GM17 (M17 supplemented with 0.5% glucose) and M17 broth with 0.5% lactose contained 0.3% bile (Bile) or not (Control). Values were determined by absorbance at 620 nm after 24 h incubation; all values are the means of two trials. –: Non-growth. Data were extracted and summarized from Kimoto et al. (2000).

survive the passage through a digestive system with typical conditions such as low pH in the stomach and the presence of bile in the intestine. However, several works showed the possibility of the presence of lactococci in the flora of the human or animal gastrointestinal tract[7,8,23,40]. In a previous study[18], the ability of a *Lactococcus* strain to pass through the gastrointestinal tract after administration to mice was examined by using an antibiotic method. Strains N7 and *Lb. acidophilus* NCFB 1748 (National Collection of Food Bacteria, England), as a reference for probiotic bacteria[28], were used for survival tests in vivo. Rifampicin-resistant variants of strains N7 and NCFB 1748 were administered to mice, and their recovery in feces was examined (Fig. 1). Before administration, no colonies were detected in plates containing rifampicin in these cases. After administration at 12, 24, 48, and 72 h, the rifampicin-resistant strain N7 appeared at levels of 10^6, 10^3, 10^2, and 0 cfu/g, respectively. Under the same conditions, the rifampicin-resistant strain NCFB 1748 was detected at levels of 10^6, 10^4, 10^2, and 0 cfu/g, respectively. These results indicate that the strain N7 could overcome specific conditions in the gastrointestinal tract of mice and reach the intestine alive after ingestion, but could not colonize. In the study, the number of viable cells reaching the mouse intestine in the first 24-h after strain N7 ingestion ranged from 10^3 to 10^6 cfu/g. It seems feasible to obtain elevated levels of viable strain N7 in the intestine by the administration of high numbers of the organisms. If administered daily, a considerable population of viable strain N7 may exist continually in the intestine. These results also indicate that the survival rate in mice of *Lactococcus* strain N7 was comparable to that of *Lb. acidophilus* strain NCFB 1748. It should be borne in mind, however, that such comparability between these two strains in the survival rate might not be the case for humans since strain NCFB 1748 is of human origin and its tolerance in humans is confirmed.

2. Adhesion to intestinal cells

Adhesion to intestinal surfaces is an important property for probiotic strains, since intestinal attachment is important for colonization on the gastrointestinal tract for many bacterial species. Adhesion has been mainly studied in vitro by using epithelial cell lines of human origin, since it is difficult to investigate bacterial adhesion in vivo. One of the cell lines often used is Caco-2 cell line[1,4,27].

Nine *Lactococcus* strains and two *Lactobacillus* strains were examined for their ability to adhere to Caco-2 cells (Table 2). *Lb. johnsonii* La1 (Nestec Culture Collection, Lausanne, Switzerland) and *Lb. acidophilus* NCFB 1748 were used as a positive control[1] and as a negative control[4], respectively. Six out of the nine *Lactococcus* strains adhered to Caco-2 cells to various extents. Strain N7 described above could not adhere to the Caco-2 cells. High attachment was observed with *Lc. lactis* subsp. *lactis* strain 527 as well as with *Lactobacillus* strain La1. Scanning electron micrographs clearly illustrate that the cells of strain 527 closely associated with the microvilli of Caco-2 cells (Fig. 2). The results were not in agreement with other reports that showed the host specificity of bacterial strains on the adhesion to the cells. For example, it was reported that isolates from plant materials, cultured milk and cheese did not adhere to epithelial cells of pigs and calves cultured in vitro[32]. However, the adhesion property of *Lc. lactis* subsp. *cremoris* ARH to Caco-2

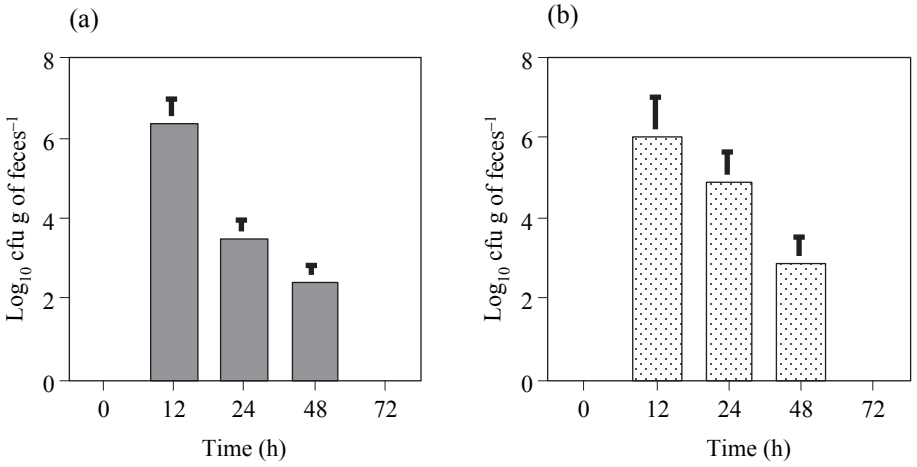

Fig. 1. Mean concentration of the populations of rifampicin-resistant lactic acid bacteria in fecal samples of mice

Standard error of mean (n = 3) is indicated as error bars. (a): *Lactococcus lactis (Lc.)* subsp. *lactis* biovar *diacetylactis* N7, (b): *Lactobacillus (Lb.) acidophilus* NCFB 1748. Reproduced from Kimoto et al. (2003) with permission of NRC Research Press.

cells was reported[27]. Strain 527, which adhered to Caco-2 cells, tolerated low pH and bile[15], indicating that it can pass through the digestive tract alive and colonize in the intestine. Further study with humans will be needed.

3. Cholesterol-removing ability

A high level of cholesterol in blood is generally considered to be a risk factor for cardiovascular disease. Therefore, decreasing serum cholesterol levels is important to prevent the disease. The effects of ingestion of probiotic bacteria such as *Lb. acidophilus* on serum cholesterol levels have attracted much interest. It has been reported that a culture of *Lb. acidophilus* actively taking up cholesterol from laboratory media would function in vivo to exert a hypocholesterolemic effect[6]. For example, *Lb. acidophilus* ATCC 43121 (the American Type Culture Collection, Rockville, MD) can incorporate some of the cholesterol taken up from media into the cellular membrane during growth[35] and the strain has beneficially influenced serum cholesterol levels in pigs[3,6]. Several other reports have been published on cholesterol removal from

Table 2. Results of in vitro adhesion tests conducted on lactococci on the human Caco-2 cell line

Strains	Result
Lactococcus lactis subsp. *lactis*	
ATCC 19435	–
712	+
527	++
1061	+
Lactococcus lactis subsp. *lactis*	
biovar *diacetylactis*	
ATCC 13675	+
DRC1	+
DRC2	–
N7	–
8W	+
Lactobacillus johnsonii	
Nestec La1	++
Lactobacillus acidophilus	
NCFB 1748	–

–: No adhesion (*Lactobacillus acidophilus* NCFB 1748 as a reference strain), +: low adhesion, ++: high adhesion (*Lactobacillus johnsonii* Nestec La1 as a reference strain). (Kimoto et al. 1999).

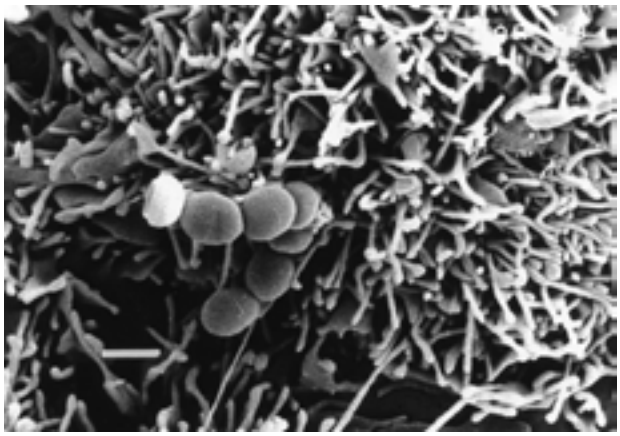

Fig. 2. Scanning electron micrograph of differentiated Caco-2 cell monolayer with adhering *Lc. lactis* subsp. *lactis* 527

Bar represents 1 μm.

Table 3. Cholesterol-removing ability and growth of lactococci[a)]

Strains	Cholesterol		
	Removed (μg mL^{-1})	Removal (%)	Growth (A620)[b)]
Lactococcus lactis subsp. *lactis*			
527	37.7	53.9	1.08
G50	62.0	88.6	1.53
H48	60.7	86.7	1.74
Lactococcus lactis subsp. *lactis*			
biovar *diacetylactis*			
N7	68.1	97.3	1.56
ATCC 13675	21.7	31.0	0.47
DRC1-F	41.0	58.6	1.42
8W	55.0	78.6	0.31
Lactobacillus acidophilus			
ATCC 43121	37.0	52.9	1.34

a): Cells were incubated for 24 h at 37ºC in GM17-THIO broth for lactococci or MRS broth for *Lactobacillus* strain supplemented with 0.2% sodium taurocholate and cholesterol (final concentration in broth = 70 μg/mL).
b): The growth of each strain was measured after culture as absorbance at 620 nm.
All values are the means of two trials in duplicate. (Kimoto et al. 2002).

laboratory media containing bile and a cholesterol source in lactobacilli.

The cholesterol-removing ability of lactococci was assessed (Table 3). All seven strains of lactococci tested had the ability to remove cholesterol from laboratory media during growth. Among the strains tested, strain N7 could remove as much cholesterol as *Lb. acidophilus* ATCC 43121. So far, it has been reported that lactococci could bind cholesterol in 60% ethanol solution[10]. However, in that study, the growth of lactococci was not considered because lactococci cannot grow in 60% ethanol solution. We focused on cholesterol removal by lactococci during growth as reported in lactobacilli[17]. In strain N7, the amount of cholesterol removed increased concomitantly with the growth (Fig. 3). This result indicates that strain N7 might use cholesterol in media during growth.

The mechanism by which lactic acid bacteria remove cholesterol from laboratory media has been studied. It has been reported that cholesterol removal by some lactobacilli was only due to a disruption of the cholesterol micelles caused by the deconjugation and precipitation of cholesterol with the free bile salts as the pH of the media dropped by acid production during growth[22]. It was confirmed that strain N7 and other lactococci tested did not produce cholic acid from sodium taurocholate. This suggested that cholesterol removal by the lactococci tested was not related to the precipitation of cholesterol with free bile salts.

There are two possible mechanisms underlying the ability of lactococci to remove cholesterol from media. One is adhesion of the cholesterol to the cell surface. It has been suggested that binding of cholesterol to the lactic acid bacterial cells may be a physical phenomenon and be related to the cell wall[10]. Another possible mechanism is an assimilation of cholesterol by the cells. It was reported that some strains of *Lb. acidophilus* incorporated some of the cholesterol into the cellular membrane[35]. Similar mechanisms should be involved in the cholesterol removal by *Lc. lactis* subsp. *lactis* bv. *diacetylactis* N7. It is evident some cholesterol bound to the strain N7 cells because even the heat-killed cells of strain N7, which cannot take up cholesterol, could remove it from media (Fig. 4). Moreover, the amount of cholesterol removed by the cells during growth (i.e., growing cells) was significantly ($P < 0.05$) higher than that removed by heat-killed and resting cells (Fig. 4). The difference in the amounts of choles-

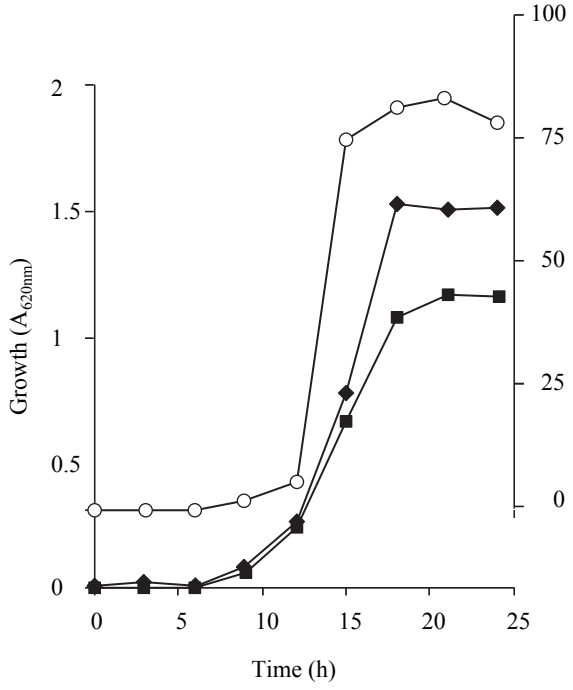

Fig. 3. Cholesterol removal (○) by and growth in GM17-THIO broth containing sodium taurocholate (■) and sodium taurocholate plus cholesterol (◆) of *Lc. lactis* subsp. *lactis* bv. *diacetylactis* N7

Values are the means of two independent experiments performed in duplicate. Reproduced from Kimoto et al. (2002) with permission of the American Dairy Science Association.

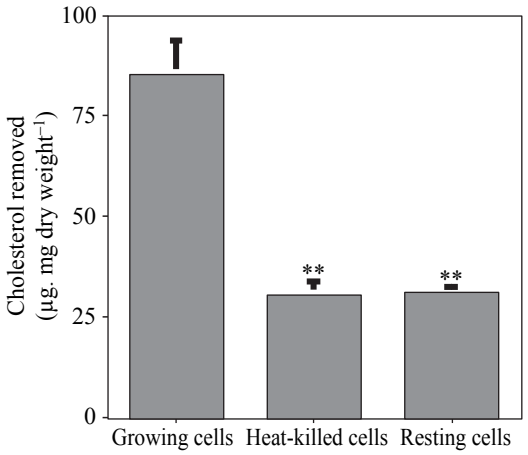

Fig. 4. Cholesterol removal by growing, heat-killed and resting cells of *Lc. lactis* subsp. *lactis* bv. *diacetylactis* N7

Cell mass was determined from a standard curve relating optical density to dry weight to calculate relative amounts of cholesterol removed (μg/mg dry weight). Cells that removed cholesterol from the media during growth for 24 h at 37ºC were designated as growing cells. The heat-killed cells of strain N7 were prepared by autoclaving for 15 min at 121ºC and were suspended in GM17-THIO broth containing sodium taurocholate plus cholesterol. Resting cells were suspended in 0.05 M phosphate solution. These cell suspensions were incubated at 37ºC for 24 h. Values are means ± SD of two independent experiments performed in duplicate. **: Significantly different from growing cells, $P < 0.01$. Reproduced from Kimoto et al. (2002) with permission of the American Dairy Science Association.

terol removed between viable cells and heat-killed cells suspension should reflect the incorporation of cholesterol into the cells during the growth. Thus, it seems that strain N7 can remove cholesterol from media both by binding of cholesterol to the cells and by the uptake of cholesterol into the cells during growth. It is likely that the mechanisms of cholesterol removal by strain N7 applies to those of other lactoccoci tested.

The hypocholesterolemic effect of strain N7 was actually shown in rats by the administration experiments[25].

4. Immune modulation

Several probiotics are claimed to have beneficial effects on a host's immune system. In vitro experiments using immunocompetent cells, experimental animal model studies and human trials have been done with some lactic acid bacteria. For example, some lactobacilli and bifidobacteria have been found to enhance immune responses, such as T-cell proliferation in vitro[30] and immunoglobulin (Ig) A production in mice[37]. With increasing numbers of allergic patients in recent years, there are several reports[41] that certain *Lactobacillus* strains suppressed IgE antibody production which relates to type-I allergy such as food and pollen allergy. In human trials with *Lactobacillus rhamnosus* strain GG[11,12], the strain ameliorated symptoms in infants with atopic eczema.

One of the mechanisms by which probiotics modulate the immune response is improvement of the unbalanced response in the immune system via cytokine production. Cytokines produced by immunocompetent cells, such as antigen-presenting cells and helper T (Th) cells, play significant roles in the immune system. Th cells are classified into two categories, type 1 Th cells (Th1 cells) and type 2 Th cells (Th2 cells)[33]. Th1 cytokines, such as interferon (IFN)-γ and interleukin (IL)-2, enhance cell-mediated immunity, and Th2 cytokines, such as IL-4, IL-5 and IL-6 enhance humoral immunity[33]. It is well known that IL-12 has an ability to induce the development of Th1 cells[45]. Thus, profiling cytokine productions of immunocompetent cells induced by lactic acid bacteria is quite important in order to characterize their immunomodulatory effects.

An in vitro experiment to screen fifteen strains of lactococci by their ability to induce cytokine production in a murine macrophage cell line, J774.1 as a model of antigen-presenting cells was carried out (Fig. 5). Among the lactococcal strains tested, *Lc. lactis* subsp. *lactis* G50 induced the highest cytokine production (IL-12, IL-6 and TNF-α) in cultured macrophage. Next, the in vivo effects of oral administration of lactococci on cytokine production in mice were examined (Fig. 6). IL-12 production in the spleen cells was more enhanced in the group fed strain G50 than it was in the control group. The production of IFN-γ, a Th1 cell-associated cytokine, by the spleen cells from mice fed strain G50 was higher than the production by the spleen cells of control mice. These results indicate that strain G50 shows an immune modulation in vivo and that oral administration of strain G50 enhances the Th1-type immune response.

It is widely accepted that the balance between Th1 and Th2 is critical for various diseases in terms of immunological status. Type-I allergy is commonly thought to be caused by the antigen-specific IgE antibody induced by a Th2-mediated immune response[46]. It has been reported

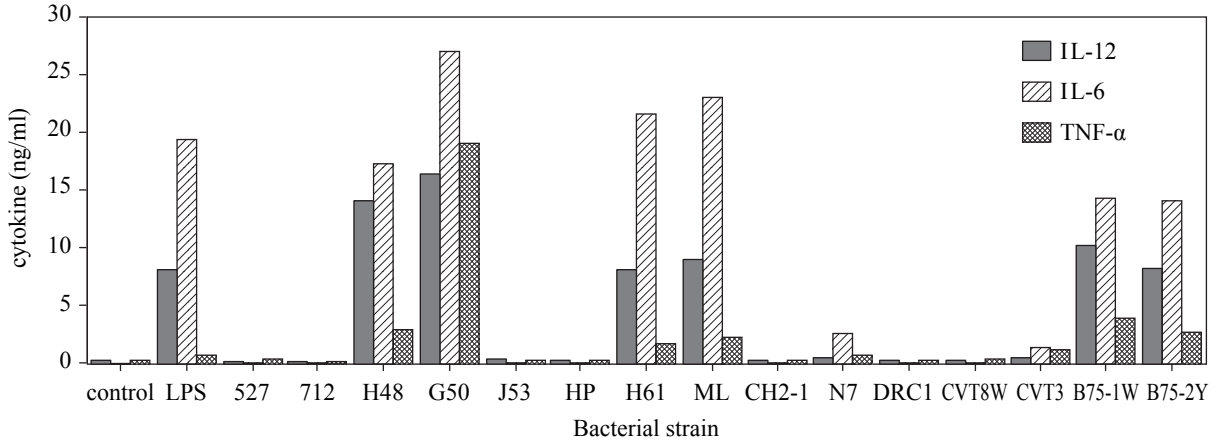

Fig. 5. Effect of lactococci on IL-12p40, IL-6 and TNF-α production by murine macrophage-like cell line J774.1 cells

J774.1 cells (5×10^5 cells/mL) were cultured with lactococci for 24 h. Production of cytokine in culture supernatant was detected by enzyme-linked immunosorbent assay. Data are representative of two separate experiments.
Strains 527, 712, H48, G50, and J53: *Lc. lactis* subsp. *lactis;* strains HP, H61, ML, and CH2-1: *Lc. lactis* subsp. *cremoris*; strains N7, DRC1, CVT8W, CVT3, B75-1W, and B75-2Y: *Lc. lactis* subsp. *lactis* bv. *diacetylactis*. Reproduced from Kimoto et al. (2004) with permission of Center for Academic Publications Japan.

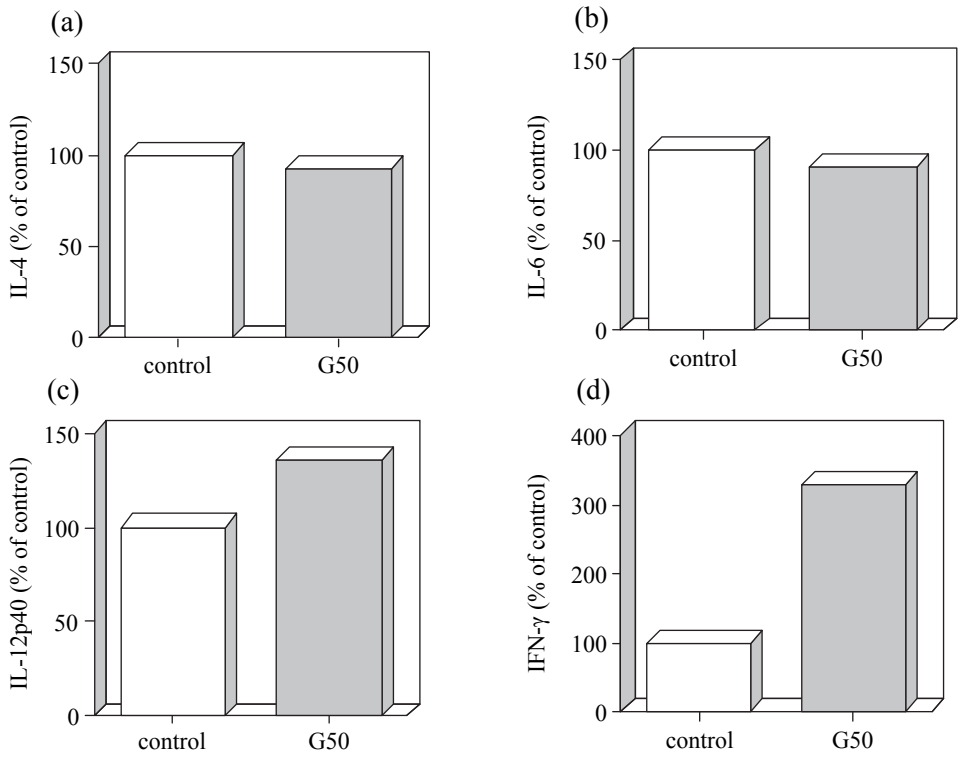

Fig. 6. Cytokine levels in spleen cells after oral exposure to *Lc. lactis* subsp. *lactis* G50 (1×10^9 cfu in 10% skim milk/mouse/day) for 7 days

Control mice were fed 10% skim milk only. Data are representative of three experiments. (a): IL-4, (b): IL-6, (c): IL-12p40, (d): IFN-γ. Reproduced from Kimoto et al. (2004) with permission of Center for Academic Publications Japan.

that enhancement of the Th1 response and suppression of the Th2 response, is a key factor to inhibit overproduction of the IgE antibody[38]. Th2 response is also known to induce the IgG1 antibody[34]. The effect of oral administration of strains G50 and *Lb. acidophilus* NCFB 1748, a reference strain, on antibody response was investigated to estimate Th1/Th2 balance in vivo. The total IgE antibody level in serum from ovomucoid (OVM), a potent egg allergen, immunized mice was significantly suppressed by oral administration of strain G50 to mice (Fig. 7). The OVM- specific IgE and IgG1 antibody levels tended to be suppressed in the group treated with strain G50. These results indicate that the Th2 response was apparently suppressed in the group treated with strain G50. This should be caused by the fact that strain G50 enhanced the Th1 response. In the case of the group administered *Lb. acidophilus* NCFB 1748, the IgE antibody production in serum was somewhat (not significantly) suppressed.

For lactic acid bacteria including lactococci, some factors that affect immune modulation have been studied. It was reported that slime products produced by *Lc. lactis*

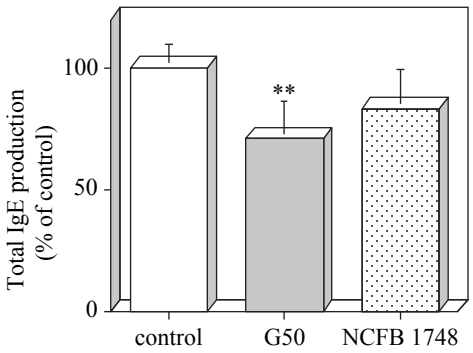

Fig. 7. Effect of oral administration of *Lc. lactis* subsp. *lactis* G50 and *Lb. acidophilus* NCFB 1748 on ovomucoid-induced total IgE antibody production in serum

Data are shown as means ± SD. Asterisks indicate values significantly different from those of the control (**: $P < 0.01$). Reproduced from Kimoto et al. (2004) with permission of Center for Academic Publications Japan.

subsp. *cremoris* showed B-cell mitogenic activity[20]. In lactobacilli, it was reported that the cell wall and cytoplasm fraction could induce cytokine production, such as IL-6 and TNF-α, in macrophage cell line RAW264, as could the whole bacterial cells[43]. *Lactococcus* strain G50 described above did not produce slime products. Induction of cytokine production in clonal macrophage by the heat-killed cells of strain G50 was observed[19], indicating that it is not critical whether cells are viable or nonviable (heat-killed) for the immunomodulatory effects of strain G50 although strain G50 was proved to reach the intestine alive by using mice (unpublished data). In strain G50, cell wall components, which constitute heat-killed cells, may be a factor for immune modulation by this strain. It has been reported that CpG[24] and AT motifs[21] in bacterial DNA are responsible for enhancement of the immune system via Toll-like receptor 9 present on immunocompetent cells[42]. CpG and AT motifs in lactococci have not been studied yet. Once effective probiotic factors are identified, the fraction including active components can be concentrated for enhancing the activity. Furthermore, an effective screening method for probiotic bacteria should be developed by using probiotic factors. The new method must lead to reducing time and efforts to screen probiotic bacteria. Probiotic factors in lactococci should be analyzed.

5. Other probiotic properties of lactococci

Some studies on the possible use of lactococci as probiotics have been carried out. It was reported that a *Lactococcus* strain administered to mice inhibited harmful intestinal bacteria[7]. Strain N7 that had cholesterol-removing ability had an antimicrobial activity to some intestinal bacteria[16] and had the ability to survive in the intestine[18]. Thus, strain N7 might influence the intestinal microbiota. Live strain N7 in the intestine may exert two beneficial effects such as antimicrobial activity and cholesterol-removing ability. Antimutagenic activity of lactococci[9] was reported. *Lc. lactis* subsp. *lactis* can produce γ-aminobutyric acid (GABA), a hypotensive agent, as do *Lactobacillus* strains[36].

Conclusion

Our data and the other studies clearly show the beneficial properties of lactococci for health and lactococci are potential probiotics. However there is a question whether probiotic properties of lactococci are superior to those of lactobacilli and bifidobacteria. Our data showed that the probiotic activities depend not on genera but on strain. *Lactococcus* strain 527 adhered well to Caco-2 cells as well as did *Lb. johnsonii* La1, a positive control. *Lactococcus* strain N7 could remove as much cholesterol as did *Lb. acidophilus* ATCC 43121. *Lactococcus* strain G50 had immunomodulatory activity such as suppression of IgE production while *Lb. acidophilus* NCFB 1748 did not. *Lactococcus* strains N7 and 527 have been used to manufacture dairy products in Japan for 50 years. Therefore, these are proved to be safe and practical candidates of new probiotic strains. Strain G50 was isolated from raw grass and its safety should be severely assessed to use as a probiotic strain. With all these strains, their effectiveness should be further proved by controlled human trials in accordance with the Declaration of Helsinki. Probiotic lactococci with scientific evidence should contribute to the development of a wide variety of new dairy products with health claims. Consequently, the potential aspects of lactococci as probiotics should be more focused in the screening of new probiotic strains.

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