J. Microbiol. Biotechnol. (2007), **17**(8), 1227–1235

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY

Cancer Chemopreventive Effects of Lactic Acid Bacteria

KIM, JONG-EUN[1], JI YEON KIM[2], KI WON LEE[3], AND HYONG JOO LEE[1]*

[1]*Department of Agricultural Biotechnology, Seoul National University, Seoul 151-742, Korea*
[2]*Nutrition and Functional Food Headquarters, Korea Food and Drug Administration, Seoul 122-704, Korea*
[3]*Department of Bioscience and Biotechnology, Konkuk University, Seoul 143-701, Korea*

Received: May 2, 2007 Accepted: June 30, 2007

Abstract Lactic acid bacteria (LAB) provide several potential health and nutritional benefits, including improving the nutritional value of food, controlling serum cholesterol levels, and controlling some types of cancer. Numerous *in vitro*, *in vivo*, human, and epidemiological studies have provided evidence of the chemopreventive effects of LAB on colon, bladder, liver, breast, and gastric cancers. These effects act *via* diverse mechanisms, including alteration of the gastrointestinal microflora, enhancement of the host's immune response, and antioxidative and antiproliferative activities. This review discusses the recent progresses on the chemopreventive effects of LAB on specific cancer types and the underlying molecular mechanisms.

Keywords: Lactic acid bacteria, cancer, chemoprevetion

Cancer is one of the leading causes of death worldwide [83], and carcinogenesis has been the subject of intense experimental, epidemiological, and clinical researches at the molecular, cellular, tissue, and clinical levels during the past two decades. However, the overall mortality rates of cancer have not declined significantly [53], and this has prompted a recent focus on reducing its incidence and the associated mortality rates. Because many human cancers are caused from preventable factors, such as infection, inflammation, smoking, and diet, preventive strategies might be the most effective to reduce cancers [52]. A proper diet may be one of the critical strategies for reducing the risk of cancer, with high consumptions of fruits and vegetables [85].

Lactic acid bacteria (LAB) have been shown to be an effective chemopreventive food ingredient against many cancer types. Over at least 4,000 years, LAB have been used to ferment foods such as cheese, yoghurt, and *kimchi* [18, 49, 51, 65]. LAB are described as Gram-positive, nonsporing, nonrespiring cocci or rods that produce lactic acid as the major end product during the fermentation of carbohydrates. The most common LAB genera in food fermentations are *Carnobacterium*, *Enterococcus*, *Lactobacillus* (*Lcb*), *Lactococcus* (*Lcc*), *Leuconostoc* (*Leu*), *Oenococcus*, *Pediococcus*, *Streptococcus* (*S*), *Tetragenococcus*, and *Weissella*. The genus *Bifidobacterium* (*Bif*) is unrelated to LAB phylogenetically, and *Bifidobacterium* species use a unique metabolic pathway for sugar metabolism. However, *Bifidobacterium* species are often considered to be LAB because they play a probiotic action by living in the gastrointestinal tract of humans and animals [84].

LAB provide several potential health and nutritional benefits, including improving the nutritional value of food, controlling gastrointestinal infections, improving digestion of lactose, controlling serum cholesterol levels, and controlling some types of cancer. These health benefits derive from a diverse range of biological activities and mechanisms [55]. This review focuses on the health-promoting benefits of LAB related to cancer prevention, the recent literature on the effects of LAB on specific cancer types, and the molecular components that underlie these effects.

Cancer-preventive Effects of LAB on Specific Cancer Types

Colorectal Cancer

Colorectal cancer is the second leading cause of cancer deaths in the United States for men and women combined. The increasing popularity of the Western meat-rich diet in Korea has increased the risk of colorectal cancer among Koreans [35]. Most researches that have investigated the relationship between LAB and cancer have concentrated on colorectal cancer. *Bifidobacterium* species decrease the growth rate and increase differentiation by increasing the activity of differentiation-related enzymes such as dipeptidyl peptidase IV and alkaline phosphatase in the HT-29 human colon adenocarcinoma cell line [5]. Fecal water from a

*Corresponding author
Phone: 82-2-880-4853; Fax: 82-2-873-5095;
E-mail: leehyjo@snu.ac.kr

healthy male who received *Lcb. plantarum* treatments exhibited significantly reduced DNA damage in the HT-29 human colon adenocarcinoma cell line [7]. In our laboratory, the cytoplasmic fraction of *Lcc. lactis* ssp. *lactis* inhibited the proliferation of the SNUC2A human colon cancer cell line by inducing S-phase cell-cycle arrest [39].

In most *in vivo* studies, the anticancer effects of LAB have been evaluated in models in which colon cancer was chemically induced. Kulkarni and Reddy [45] reported that azoxymethane induced colonic aberrant crypts in male F344 rats fed a high-fat diet, whereas a group fed with lyophilized cultures of *Bifidobacterium* species showed 50% reduced rate compared with a control group. Rowland *et al.* [75] suggested that this result was due to a decrease in the β-glucuronidase activity and ammonia concentration in the rat feces. Singh *et al.* [82] also showed that the Ras mutation and ornithine decarboxylase activity decreased. Reddy and Rivenson [73] demonstrated the effects of *Bif. longum* on 2-amino-3-methylimidazo[4,5-f]quinoline (IQ)-induced colon carcinogenesis: F344 rats exposed to 0% and 0.5% lyophilized cultures of *Bif. longum* with 125 ppm IQ for 58 weeks exhibited a significantly lower incidence of colorectal tumors. 1,2-Dimethylhydrazine (DMH) also induces colon carcinogenesis, and *Lcb. acidophilus* reduced the incidence of colon tumors in DMH-treated F344 rats by 48% compared with a DMH-only group [20]. Fujino [16] also demonstrated that the incidence of colon tumors was reduced by 24% in DMH-treated mice that received a dietary supplement containing several LAB cultures compared with a DMH-only group.

In clinical tests, the number of *Bifidobacterium* species was significantly reduced in the fecal intestinal flora of patients with colon adenoma [44]. The administration of *Lcb. casei* can prevent the development of colorectal cancer, with a daily intake of live *Lcb. casei* suppressing atypia of colorectal tumors in 398 men and women who were free from tumors and who had had at least two colorectal tumors removed [29]. Rafter *et al.* [72] performed a 12-week randomized, double-blind, placebo-controlled trial of a foodstuff containing inulin, *Lcb. rhamnosus* GG, and *Bif. lactis* Bb12 in 37 colon cancer patients and 43 polypectomized patients. They found that fecal flora exhibited increased *Bifidobacterium* and *Lactobacillus* species and decreased *Clostridium* (*C*) *perfringens*. Colorectal proliferation and fecal-water-induced DNA damage of HT-29 human colon cancer cells were decreased. Moreover, epithelial barrier function was improved in polypectomized patients.

Bladder Cancer

The bladder is the fourth leading cancer-afflicted organ among men in the United States (after prostate, lung, and colon cancers) [30]. Smoking, working in rubber, chemical, and leather industries, and chronic bladder infection are the main causes of this type of cancer. The incidence of bladder cancer is nearly four times higher in men than in women, which may be attributable to the gender difference in the smoking rate [79]. This type of cancer is relatively easy to treat, but also frequently recurs and regrows more malignantly [87].

Seow *et al.* [80] demonstrated the antiproliferative activities of two types of lactobacilli on two bladder cancer cell lines: both *Lcb. rhamnosus* GG and *Lcb. casei* strain *Shirota* inhibited cell growth in the MGH and RT112 bladder cancer cell lines. Bladder cancer was induced in that study by inoculating MB49 cells in C57BL/6 mice, which were then administered *Lcb. rhamnosus* GG. The group fed *Lcb. rhamnosus* GG exhibited significantly reduced tumor formation and growth. The authors attributed this effect to immunomodulation, as indicated by increased spleen CD3, CD4, and CD8a T lymphocytes and natural killer (NK) cells [54]. An epidemiological study found that habitual intake of *Lcb. casei* strain *Shirota* reduced the risk of bladder cancer [63]. LAB are also effective at reducing the rate of recurrence. Furthermore, the 50% recurrence-free interval of bladder cancer was increased 1.8-fold by the oral administration of *Lcb. casei* in patients who had received transurethral resection of bladder tumors [2].

Liver Cancer

Cancer originating in the liver is uncommon in North America and Western Europe, whereas in Korea the liver is the third leading site of diagnosed cancers (after gastric and lung cancers) [4]. Hepatic metastases are most common from cancers of the gastrointestinal tract, lung, and breast, due to the large blood flow through the liver. Hepatic disease usually develops from hepatitis, which is caused by liver damage due to viruses, alcohol, and chemical compounds (*e.g.*, aflatoxins, nitrosamines, and azo compounds) where the hepatitis leads to cirrhosis and finally to cancer. Therefore, liver cancer can be prevented by reducing liver damage [62].

There is considerable evidence that LAB reduce liver damage. Alteration of the gastrointestinal microflora to LAB and the antioxidant effects of LAB can reduce liver damage *via* the mechanisms described above [12]. Intragastric feeding of *Lcb. rhamnosus* (2×10^{10} CFU/ml) reduced endotoxemia and alcohol-induced liver injury in the rat [61], and administration of *Lcb. acidophilus* reduced carbontetrachloride- and tert-butyl hydroperoxide-induced liver damage and β-glucuronidase activity. *Lcb. acidophilus* is more effective than a commercial hepatoprotective agent, dimethyl diphenyl bicarboxylate, at protecting against liver damage [25]. A mixture of *Lcb. rhamnosus* LC705 and *Propionibacterium freudenreichii* protected the liver from aflatoxin: the aflatoxin exposure was reduced by 55% in 45 healthy young men taking a dietary supplement containing LAB compared with another

Table 1. Anticancer effects of LAB.

Target organ	Model	Strain	Effect	Reference
Colon	HT-29[a]	*Bifidobacterium* species	Reduce cell growth Increase differentiation	[5]
	HT-29	*Lactobacillus plantarum*	Reduce DNA damage	[7]
	SNUC2A[b]	*Lactococcus lactis* ssp. *lactis*	S-phase cell cycle arrest	[40]
	Azoxymethane-induced colorectal cancers in male F344 rats	*Bifidobacterium longum*	Reduce aberrant crypts Reduce β-glucuronidase Reduce ammonia concentration Reduce Ras mutation Reduce ODC[c] activity	[45, 75, 82]
	IQ-induced colorectal cancers in male F344 rats	*Bifidobacterium longum*	Reduce tumor incidence	[73]
	DMH-induced colorectal cancers in male F344 rats	*Lactobacillus acidophilus*	Reduce tumor incidence	[16, 20]
	At least 2 colorectal tumors removed from 398 men and women	*Lactobacillus casei*	Reduce atypia	[29]
	37 colon cancer patients and 43 polypectomized patients	*Lactobacillus rhamnosus* GG *Bifidobacterium lactis* Bb12	Change fecal microflora Reduce proliferation Reduce fecal-water-induced DNA damage	[72]
Bladder	MGH[d] RT112[e]	*Lactobacillus rhamnosus* GG *Lactobacillus casei* strain *Shirota*	Reduce cell growth	[80]
	MB49[f]-injected C57BL/6 mice	*Lactobacillus rhamnosus* GG	Increase in spleen CD3, CD4, and CD8a T lymphocytes and NK cells	[54]
	180 bladder cancer patients	*Lactobacillus casei* strain *Shirota*	Reduce bladder cancer	[63]
	Patients with transurethral resection of the bladder tumor	*Lactobacillus casei*	Increase the 50% recurrence-free interval 1.8-fold	[2]
Liver	Alcohol-fed male Wistar rats	*Lactobacillus rhamnosus* GG	Reduce endotoxemia Reduce liver injury	[61]
	Carbontetrachloride and tert-butyl hydroperoxide-fed ICR mice	Lactobacillus acidophilus	Reduce liver damage Reduce β-glucuronidase	[25]
	45 men	*Lactobacillus rhamnosus* LC705	Reduce aflatoxin exposure	[14]
Breast	MCF7[g]	*Bifidobacterium infantis* *Bifidobacterium bifidum* *Bifidobacterium animalis* *Lactobacillus acidophilus* *Lactobacillus paracasei*	Reduce cell growth	[6]
	4T1[h]-injected BALB/c mice	*Lactobacillus helveticus* R389	Reduce tumor growth Increase cytokines	[13]
	133 breast cancer patients and 289 healthy controls	Fermented milk products	Reduce breast cancer	[88].
Stomach	Coculture of LAB and *Helicobacter pylori* in Brucella agar plates	*Lactobacillus plantarum* MLBPL1	Reduce growth of *Helicobacter pylori*	[74]
	Helicobacter pylori-infected C57BL/6 mice	*Lactobacillus casei* strain *Shirota*	Reduce colonization of *Helicobacter pylori*	[81]

[a]HT-29: human colon cancer cell line.
[b]SNUC2A: human colon adenocarcinoma cell line.
[c]ODC: ornithine decarboxylase.
[d]MGH: human bladder cancer cell line.
[e]RT112: human bladder cancer cell line.
[f]MB49: mouse bladder cancer cell line.
[g]MCF7: human breast cancer cell line.
[h]4T1: mouse mammary tumor cell line.

45 men taking a placebo [14]. Therefore, preventing liver damage by LAB may help to reduce the incidence of liver cancer. However, there have also been a few reports on a direct correlation between LAB and liver cancer, and hence this relationship needs to be studied further.

Breast Cancer

Breast cancer is the most-diagnosed female cancer in the United States [30]. In Korea, it is the second most common type of cancer after gastric cancer, and its incident rate is steadily increasing, which may be due to the increasing popularity of the Western diet (as for colorectal cancer) [43]. Fermented milk containing five LAB (*Bif. infantis*, *Bif. bifidum*, *Bif. animalis*, *Lcb. acidophilus*, and *Lcb. paracasei*) inhibited the growth of the MCF7 breast cancer cell line [6]. De Moreno de LeBlanc *et al.* [13] employed an *in vivo* breast cancer model in which BALB/c mice received subcutaneous injections of 4T1 mouse mammary adenocarcinoma cells, and found that feeding the mice with fermented milk containing *Lcb. helveticus* R389 reduced tumor growth and increased cytokines such as interleukin (IL)-10 and IL-4. In a case-control study in The Netherlands, the consumption of fermented milk products was significant less among 133 breast cancer patients than among 289 healthy controls, suggesting that LAB can prevent breast cancer [88].

Gastric Cancer

The incidence of gastric cancer has declined in the United States since 1930, which may account for the decrease in *Helicobacter* (*H*) *pylori* infections due to antibiotic treatment [30]. However, in Korea, gastric cancer remains one of the most-diagnosed cancers, which is partly due to *H. pylori* infections not decreasing because of the presence of unique eating habits such as eating same dish together [66]. Therefore, treatment of *H. pylori* is important to preventing gastric cancer in Korea. Most studies of gastric cancer related to LAB have involved their inhibitory effects against *H. pylori* [15]. However, we suggested other mechanism of how LAB can reduced gastric cancer in our study. The cytoplasmic fraction of *L. lactis* ssp. *lactis* induced apoptosis in the SNU-1 human adenocarcinoma cell line. We have also found that arginine deiminase is the active compound that induces apoptosis in this cell line [36, 41].

In summary, many studies have provided evidence that LAB can prevent different cancer types, such as colon, bladder, liver, breast, and gastric cancers (Table 1).

MECHANISMS OF ANTICANCER EFFECTS OF LAB

Alteration of the Gastrointestinal Microflora

Many genera and species of microorganisms inhabit the human gastrointestinal tract. Specific components of the gastrointestinal microflora provide health benefits, such as energy salvage, antagonism against pathogens, immune stimulation of the gut-associated lymphoid tissue, innate immunity against infections, and production of vitamins [23]. However, harmful bacteria such as *C. perfringens* can produce genotoxic, carcinogenic, and tumor-promoting components. The water in human feces exhibits genotoxicity and cytotoxicity against colon cells. Furthermore, germ-free animals exhibit much lower incidences of colorectal cancer. These findings indicate that cancer may be at least partly due to the gastrointestinal microflora.

LAB enter the gut along with consumed food and are known to have beneficial effects on the resident bacteria of the gastrointestinal microflora [86]. They compete with other bacteria in the human body by producing inhibitory compounds (*e.g.*, organic acids, hydrogen peroxide, bacteriocins, and reuterin) or competitively adhering to the epithelium. LAB can provide many health benefits if they can overcome harmful bacteria, and hence are called probiotics, which have been defined as "living microorganisms, which upon ingestion in certain numbers exert health benefits beyond inherent basic nutrition" [17].

The anticancer effects of LAB associated with alterations to the gastrointestinal microflora stem from mechanisms including antimutagenic effects and reducing harmful bacteria. Many mutagenic compounds are present in the Western meat-rich diet, and they can bind to the gastrointestinal tract, whereas LAB reduce the mutagenicity observed after exposure to the bacterial strains [46]. Orrhage *et al.* [64] reported on the binding capacity of eight human gastrointestinal or LAB strains for mutagenic heterocyclic amines formed during the cooking of protein-rich food. The binding appears to be a physical phenomenon, mostly due to a cation-exchange mechanism, and it has been suggested that cell-wall peptidoglycans and polysaccharides are the two most important underlying elements [92]. It has also been shown that oral administration of *Lcb. acidophilus* inhibits the DNA damage induced by *N*-nitro-*N*-nitrosoguanidine, using the comet assay in both rat gastric and colonic mucosa [71].

Some microorganisms such as bacteroides, eubacteria, and clostridia produce metabolic end products that are considered to be carcinogenic and genotoxic compounds (*e.g.*, nitrosamines, heterocyclic amines, various aglycones, some azo compounds, and ammonia) [89]. In particular, β-glucuronidase, azoreductase, and nitroreductase are involved in producing these compounds. β-Glucuronidase has wide substrate specificity and can hydrolyze many different glucuronides to carcinogenic aglycones. Nitroreductase and azoreductase reduce nitro and azo compounds to aromatic amines, which are reactive intermediates whose end products are known mutagens and carcinogens. Since LAB exhibit low levels of these enzyme activities, increasing the LAB in gastrointestinal microflora will decrease the

activities of these enzymes. Oral supplementation of human and rat diets with viable *Lcb. acidophilus* decreases bacterial β-glucuronidase, nitroreductase, and azoreductase by about 50% [19, 21]. It has also been demonstrated that *Lcb. acidophilus* reduces β-glucuronidase in human-flora-associated rats. Moreover, feeding with *Bif. longum* also reduced β-glucuronidase activity by 30%.

H. pylori are Gram-negative, spiral-shaped, microaerophilic rods that colonize the human gastric mucosa by producing urease, which hydrolyzes urea to ammonium, leading to increased stomach pH that causes inflammatory diseases such as chronic gastritis. Gastric cancer is strongly related to a transition from normal mucosa to gastritis, which eventually leads to adenocarcinoma [68]. Therefore, an international agency for research on cancer has classified *H. pylori* as a class I carcinogen. LAB prevent or decrease the growth and colonization of *H. pylori* [22, 74]. An *in vitro* study found that *Lcb. casei* strain *Shirota* reduced *H. pylori* growth rates [81]. A significant reduction of *H. pylori* colonization in the *Lactobacillus*-treated group was also observed in the antrum and body mucosa in *H. pylori*-infected C57BL/6 mice, which was accompanied by a significant decrease in the associated chronic and active gastric mucosal inflammation. Other LAB such as *Lcb. acidophilus* 4356 have also been shown to attenuate the growth of *H. pylori* both *in vivo* and *in vitro* [3, 31, 42, 47, 59].

Enhancement of the Host's Immune Response

Immunomodulation is a putative target for cancer therapy. Since Coley attempted to treat cancer patients by boosting the immune system with bacterial extracts, many researchers have attempted to cure cancer by immunomodulation [1]. Through advances in cellular and molecular immunology during the past two decades, many studies have shown that LAB enhance the host's immunoprotective system *via* mechanisms such as releasing cytokines and phagocytosis [90]. Intrapleural administration of *Lcb. casei* strain *Shirota* into tumor-bearing mice inhibited tumor growth and increased survival, because it induced the production of several cytokines such as interferon (IFN)-γ, IL-1, and tumor necrosis (TNF)-α [60]. Anti-TNF-α monoclonal antibody treatment completely abolished the antitumor effect of *Lcb. casei* strain *Shirota in vivo*. However, anti-IFN-γ and anti-IL-1β monoclonal antibodies had no effect [91]. *Lcb. casei* strain *Shirota* induced IL-12 and IFN-γ in murine splenocytes [33]. There are reports of other strains of LAB inducing cytokines and subsequently inhibiting tumors. *Bif. longum* and *Bif. animalis* induced inflammatory cytokines such as IL-6 and TNF-α [77]. Human bifidobacteria isolates induced H_2O_2, nitric oxide (NO), and IL-6 [67]. We previously found that *Lcb. plantarum* exerted the strongest effect on TNF-α, IL-6, and NO production out of six strains of major LAB found in *kimchi* (*Leu. mesenteroides*, *Leu. citreum*, *Lcb. plantarum*, *Lcb. sake*, *Bif. longum*, and *Bif. lactis*) in the RAW 264.7 murine macrophage cell line [27, 28]. We also observed that heat-killed *Lcc. lactis* ssp. *lactis* stimulated IFN-γ, IL-6, IL-12, and TNF-α in spleen cells. The cellular components of *L. lactis* ssp. *lactis* induced only TNF-α in peritoneal-exudate cells. Intraperitoneal administration of whole-cell [37] and cytoplasmic fractions of *L. lactis* ssp. *lactis* to male Balb/c mice resulted in the production of IFN-γ, IL-2, and IL-12 [38].

It has been shown that administering *L. casei* LC9018 to C57BL/6 mice enhanced the phagocytic activity of peritoneal macrophages [34]. Perdigon *et al*. [69] observed that macrophage and lymphocyte activities were enhanced in mice after administering a mixed culture of *Lcb. acidophilus* and *Lcb. casei*. This group also reported activation of peritoneal macrophages in mice after oral administration of *Lcb. casei* and *Lcb. bulgaricus*. Similar results were found for the oral delivery of *S. thermophilus* and *Lcb. acidophilus* [70] and the injection of heat-killed *Lcb. casei* to mice [76]. Lee *et al*. [50] recently demonstrated that administering the cytoplasmic fraction of *Lcb. casei* and *Bif. longum* as dietary supplements to Balb/c mice for 4 weeks enhanced the numbers of total T cells, NK cells, MHC class II+ cells, and CD4-CD8+ T cells. We have also demonstrated that oral administration of heat-killed *L. lactis* ssp. *lactis* to male Balb/c mice induced phagocytic activity [38].

Antioxidative Activity

There are multiple lines of evidence from both laboratory and clinical studies that oxidative stresses imposed by reactive oxygen species (ROS) play a key role in all stages of carcinogenesis. Several oxidants and free-radical generators are also known tumor promoters [8]. Many tumor promoters generate ROS, and the involvement of ROS (particularly H_2O_2) in the tumor promotion is supported by both *in vivo* and *in vitro* studies [24]. Therefore, dietary substances with antioxidant activities are anticipated to exert chemopreventive effects at all stages of carcinogenesis [52]. Kaizu *et al*. [32] found that *Lcb.* ssp. SBT 2028 exerted the strongest antioxidant effects out of 570 strains of LAB. Hemolysis of red blood cells was inhibited by administering the extract of *Lcb.* ssp. SBT 2028 to rats with vitamin E deficiency, suggesting that this LAB acts as a substitute for vitamin E. Lin and Yen [57] demonstrated that 19 strains of LAB exhibited antioxidant activities of 7–12% in intracellular cell-free extracts, which was due to their metal-ion-chelating and ROS-scavenging abilities. *Lcb. acidophilus* and *Bif. longum* inhibit lipid peroxidation, as demonstrated by two methods in which linoleic acid and the cell membrane of osteoblasts were used for lipid peroxidation. These strains protected against lipid oxidation by 33–46% in terms of linoleic acid peroxidation and by

22–37% in terms of cell membrane lipid peroxidation [58]. Both intact-cell and intracellular-cell-free extracts exerted antioxidant activities [56]. Heat-killed cells of *Lcb. acidophilus* 606 also exert antiproliferative activity [11], which is due to the soluble polysaccharide fraction; this fraction also exhibits potent antioxidant activity.

Antiproliferative Activity

Cancer usually is defined as "uncontrolled cell growth". The maintenance of homeostasis in normal mammalian tissues may involve a critical balance between cell proliferation and cell death, with external or internal causes upsetting this balance, resulting in cancer [26]. Therefore, strategies for keeping cells under fine control have been used to prevent cancer, whereby cancer cells are killed or their proliferation is inhibited [78]. We have screened the cytotoxicity in whole-cell, cytoplasm, and peptidoglycan fractions of 10 LAB on 11 types of cancer cell lines using the ^{3}H-thymidine incorporation assay [40]. The peptidoglycan and cytoplasm fractions as well as heat-killed whole-cell fraction of LAB exhibited significant antiproliferative activities against several cancer cell lines. In particular, the cytoplasm fractions exhibited marked direct antiproliferative activities against colon and gastric cancer cell lines, whereas the peptidoglycans inhibited the growth of colon and bladder cancer cell lines. In particular, the cytoplasm fraction of *Lcc. lactis* ssp. *lactis* mostly inhibited the proliferation of the SNUC2A human colon cancer cell line by downregulation of cyclin-dependent kinase 2 and overexpression of cyclin A [39].

Although the precise mechanisms of the anticancer effects of LAB remain unclear, several possible mechanisms include alteration of the gastrointestinal microflora, enhancement of the host's immune response, and antioxidative and antiproliferative activities (Fig. 1).

Concluding Remarks

LAB have traditionally been used to enhance the preservation properties, flavors, and textures of foodstuffs. The works of Metchnikoff heralded an emphasis of the health benefits of LAB. Nowadays, the functional food market is in the limelight, with many advertisements focusing on the functionality of foods [9, 10, 48]. However, some of these foods are very expensive and the safety of others is unclear. LAB may be particularly safe active ingredients of functional foods owing to their long history of use. LAB are already widely consumed in fermented foods such as yoghurt, cheese, and *kimchi*. In this review, we have described the results from studies related to the anticancer effects of LAB. However, most of these studies have only described the phenotype of the anticancer effects of LAB, and hence further investigations into the direct mechanisms and molecular targets are needed to clarify these effects. A better understanding about cancer and LAB may significantly contribute to improvements in the health of populations worldwide.

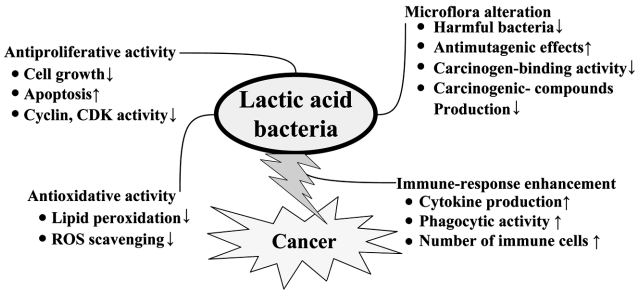

Fig. 1. Mechanisms of cancer chemopreventive effects of LAB.

Acknowledgment

This work was supported by a grant from BioGreen 21 Program, Rural Development Administration (no. 20070301-034-042) and the Korea Institute of Science and Technology Evaluation and Planning (KISTEP) for Functional Food Research and Development, Ministry of Science and Technology (no. 2007-01866), Republic of Korea.

References

1. Agha-Mohammadi, S. and M. T. Lotze. 2000. Immunomodulation of cancer: Potential use of selectively replicating agents. *J. Clin. Invest.* **105:** 1173–1176.
2. Aso, Y. and H. Akazan. 1992. Prophylactic effect of a *Lactobacillus casei* preparation on the recurrence of superficial bladder cancer. *Urol. Int.* **49:** 125–129.
3. Bae, E. A., D. H. Kim, and M. J. Han. 2000. Anti-*Helicobacter pylori* activity of *Bifidobacterium* spp. *J Microbiol. Biotechnol.* **10:** 532–534.
4. Bae, J. M., K. W. Jung, and Y. J. Won. 2002. Estimation of cancer deaths in Korea for the upcoming years. *J. Korean Med. Sci.* **17:** 611–615.
5. Baricault, L., G. Denariaz, J. J. Houri, C. Bouley, C. Sapin, and G. Trugnan. 1995. Use of HT-29, a cultured human colon cancer cell line, to study the effect of fermented milks on colon cancer cell growth and differentiation. *Carcinogenesis* **16:** 245–252.
6. Biffi, A., D. Coradini, R. Larsen, L. Riva, and G. Di Fronzo. 1997. Antiproliferative effect of fermented milk on the growth of a human breast cancer cell line. *Nutr. Cancer* **28:** 93–99.
7. Burns, A. J. and I. R. Rowland. 2004. Antigenotoxicity of probiotics and prebiotics on faecal water-induced DNA damage in human colon adenocarcinoma cells. *Mutat. Res.* **551:** 233–243.

8. Cerutti, P. A. 1985. Prooxidant states and tumor promotion. *Science* **227:** 375–381.
9. Chang, H. C., Y. D. Choi, and H. J. Lee. 1996. Molecular cloning of a β-D-galactosidase gene from *Lactococcus lactis* subsp. *lactis* 7962. *J Microbiol. Biotechnol.* **6:** 386–390.
10. Chang, J. H., J. H. Shin, I. S. Chung, and H. J. Lee. 1999. Improved menthol production using suspension cultures of *Mentha piperita* with pectinase elicitation. *J. Microbiol. Biotechnol.* **9:** 358–360.
11. Choi, S. S., Y. Kim, K. S. Han, S. You, S. Oh, and S. H. Kim. 2006. Effects of *Lactobacillus* strains on cancer cell proliferation and oxidative stress *in vitro*. *Lett. Appl. Microbiol.* **42:** 452–458.
12. Clausen, M. R. and P. B. Mortensen. 1997. Lactulose, disaccharides and colonic flora. Clinical consequences. *Drugs* **53:** 930–942.
13. de Moreno de LeBlanc, A., C. Matar, N. LeBlanc, and G. Perdigon. 2005. Effects of milk fermented by *Lactobacillus helveticus* R389 on a murine breast cancer model. *Breast Cancer Res.* **7:** R477–R486.
14. El-Nezami, H. S., N. N. Polychronaki, J. Ma, H. Zhu, W. Ling, E. K. Salminen, R. O. Juvonen, S. J. Salminen, T. Poussa, and H. M. Mykkanen. 2006. Probiotic supplementation reduces a biomarker for increased risk of liver cancer in young men from Southern China. *Am. J. Clin. Nutr.* **83:** 1199–1203.
15. Felley, C. and P. Michetti. 2003. Probiotics and *Helicobacter pylori*. *Best Pract. Res. Clin. Gastroenterol.* **17:** 785–791.
16. Fujino, T. 2001. The tumor-preventing effect of a mixture of several lactic acid bacteria on 1,2-dimethylhydrazine-induced colon carcinogenesis in mice. *Oncol. Rep.* **8:** 1073–1078.
17. Gibson, G. R. and R. Fuller. 2000. Aspects of *in vitro* and *in vivo* research approaches directed toward identifying probiotics and prebiotics for human use. *J. Nutr.* **130:** 391S–395S.
18. Gilliland, S. E. 1990. Health and nutritional benefits from lactic acid bacteria. *FEMS Microbiol. Rev.* **7:** 175–188.
19. Goldin, B. and S. L. Gorbach. 1977. Alterations in fecal microflora enzymes related to diet, age, *Lactobacillus* supplements, and dimethylhydrazine. *Cancer* **40:** 2421–2426.
20. Goldin, B. R. and S. L. Gorbach. 1980. Effect of *Lactobacillus acidophilus* dietary supplements on 1,2-dimethylhydrazine dihydrochloride-induced intestinal cancer in rats. *J. Natl. Cancer Inst.* **64:** 263–265.
21. Goldin, B. R. and S. L. Gorbach. 1984. The effect of milk and *Lactobacillus* feeding on human intestinal bacterial enzyme activity. *Am. J. Clin. Nutr.* **39:** 756–761.
22. Gotteland, M. and S. Cruchet. 2003. Suppressive effect of frequent ingestion of *Lactobacillus johnsonii* La1 on *Helicobacter pylori* colonization in asymptomatic volunteers. *J. Antimicrob. Chemother.* **51:** 1317–1319.
23. Guarner, F. and J.-R. Malagelada. 2003. Gut flora in health and disease. *Lancet* **361:** 512–519.
24. Halliwell, B. 2007. Oxidative stress and cancer: Have we moved forward? *Biochem. J.* **401:** 1–11.
25. Han, S. Y., C. S. Huh, Y. T. Ahn, K. S. Lim, Y. J. Baek, and D. H. Kim. 2005. Hepatoprotective effect of lactic acid bacteria, inhibitors of beta-glucuronidase production against intestinal microflora. *Arch. Pharm. Res.* **28:** 325–329.
26. Hanahan, D. and R. A. Weinberg. 2000. The hallmarks of cancer. *Cell* **100:** 57–70.
27. Hur, H. J., K. W. Lee, H. Y. Kim, D. K. Chung, and H. J. Lee. 2006. *In vitro* immunopotentiating activities of cellular fractions of lactic acid bacteria isolated from *kimchi* and *Bifidobacteria*. *J. Microbiol. Biotechnol.* **16:** 661–666.
28. Hur, H. J., K. W. Lee, and H. J. Lee. 2004. Production of nitric oxide, tumor necrosis factor-alpha and interleukin-6 by RAW264.7 macrophage cells treated with lactic acid bacteria isolated from *kimchi*. *Biofactors* **21:** 123–125.
29. Ishikawa, H., I. Akedo, T. Otani, T. Suzuki, T. Nakamura, I. Takeyama, S. Ishiguro, E. Miyaoka, T. Sobue, and T. Kakizoe. 2005. Randomized trial of dietary fiber and *Lactobacillus casei* administration for prevention of colorectal tumors. *Int. J. Cancer* **116:** 762–767.
30. Jemal, A., R. Siegel, E. Ward, T. Murray, J. Xu, and M. J. Thun. 2007. Cancer statistics, 2007. *CA Cancer J. Clin.* **57:** 43–66.
31. Kabir, A. M., Y. Aiba, A. Takagi, S. Kamiya, T. Miwa, and Y. Koga. 1997. Prevention of *Helicobacter pylori* infection by lactobacilli in a gnotobiotic murine model. *Gut* **41:** 49–55.
32. Kaizu, H., M. Sasaki, H. Nakajima, and Y. Suzuki. 1993. Effect of antioxidative lactic acid bacteria on rats fed a diet deficient in vitamin E. *J. Dairy Sci.* **76:** 2493–2499.
33. Kato, I., T. Yokokura, and M. Mutai. 1988. Correlation between increase in Ia-bearing macrophages and induction of T cell-dependent antitumor activity by *Lactobacillus casei* in mice. *Cancer Immunol. Immunother.* **26:** 215–221.
34. Kato, I., T. Yokokura, and M. Mutai. 1983. Macrophage activation by *Lactobacillus casei* in mice. *Microbiol. Immunol.* **27:** 611–618.
35. Kim, D. W., Y. J. Bang, D. S. Heo, and N. K. Kim. 2002. Colorectal cancer in Korea: Characteristics and trends. *Tumori* **88:** 262–265.
36. Kim, J. E., D. W. Jeong, and H. J. Lee. 2007. Expression, purification, and characterization of arginine deiminase from *Lactococcus lactis* ssp. *lactis* ATCC 7962 in *Escherichia coli* BL21. *Protein Expr. Purif.* **53:** 9–15.
37. Kim, J. Y., S. Lee, D. W. Jeong, S. Hachimura, S. Kaminogawa, and H. J. Lee. 2005. Effects of intraperitoneal administration of *Lactococcus lactis* ssp. *lactis* cellular fraction on immune response. *Food Sci. Biotechnol.* **14:** 405–409.
38. Kim, J. Y., S. Lee, D. W. Jeong, S. Hachimura, S. Kaminogawa, and H. J. Lee. 2006. *In vivo* immunopotentiating effects of cellular components from *Lactococcus lactis* ssp. *lactis*. *J Microbiol. Biotechnol.* **16:** 786–790.
39. Kim, J. Y., H. J. Woo, Y. S. Kim, K. H. Kim, and H. J. Lee. 2003. Cell cycle dysregulation induced by cytoplasm of *Lactococcus lactis* ssp. *lactis* in SNUC2A, a colon cancer cell line. *Nutr. Cancer* **46:** 197–201.
40. Kim, J. Y., H. J. Woo, Y. S. Kim, and H. J. Lee. 2002. Screening for antiproliferative effects of cellular components from lactic acid bacteria against human cancer cell lines. *Biotechnol. Lett.* **24:** 1431–1436.

41. Kim, S. Y., K. W. Lee, J. Y. Kim, and H. J. Lee. 2004. Cytoplasmic fraction of *Lactococcus lactis* ssp. *lactis* induces apoptosis in SNU-1 stomach adenocarcinoma cells. *Biofactors* **22:** 119–122.
42. Kim, T. S., J. W. Hur, M. A. Yu, C. I. Cheigh, K. N. Kim, J. K. Hwang, and Y. R. Pyun. 2003. Antagonism of *Helicobacter pylori* by bacteriocins of lactic acid bacteria. *J. Food Prot.* **66:** 3–12.
43. Kim, Y., J. Y. Choi, K. M. Lee, S. K. Park, S. H. Ahn, D. Y. Noh, Y. C. Hong, D. Kang, and K. Y. Yoo. 2007. Dose-dependent protective effect of breast-feeding against breast cancer among ever-lactated women in Korea. *Eur. J. Cancer Prev.* **16:** 124–129.
44. Kubota, Y. 1990. Fecal intestinal flora in patients with colon adenoma and colon cancer. *Nippon Shokakibyo Gakkai Zasshi* **87:** 771–779.
45. Kulkarni, N. and B. S. Reddy. 1994. Inhibitory effect of *Bifidobacterium longum* cultures on the azoxymethane-induced aberrant crypt foci formation and fecal bacterial beta-glucuronidase. *Proc. Soc. Exp. Biol. Med.* **207:** 278–283.
46. Lankaputhra, W. E. and N. P. Shah. 1998. Antimutagenic properties of probiotic bacteria and of organic acids. *Mutat. Res.* **397:** 169–182.
47. Lee, H. M. and Y. Lee. 2006. Isolation of *Lactobacillus plantarum* from *kimchi* and its inhibitory activity on the adherence and growth of *Helicobacter pylori*. *J Microbiol. Biotechnol.* **16:** 1513–1517.
48. Lee, J. E., G. J. Woo, and H. J. Lee. 1996. Tetramethylpyrazine production by immobilized culture of *Lactococcus lactis* subsp. *lactis* biovar. *diacetilactis* FC1. *J Microbiol. Biotechnol.* **6:** 137–141.
49. Lee, J. M., J. Y. Choi, J. H. Lee, H. C. Chang, D. K. Chung, J. H. Kim, and H. J. Lee. 1999. Cloning and expression of the UDP-galactose-4-epimerase gene (galE) constituting the gal/lac operon of *Lactococcus lactis* ssp. *lactis* ATCC7962. *J Microbiol. Biotechnol.* **9:** 393–397.
50. Lee, J. W., J. G. Shin, E. H. Kim, H. E. Kang, I. B. Yim, J. Y. Kim, H. G. Joo, and H. J. Woo. 2004. Immunomodulatory and antitumor effects *in vivo* by the cytoplasmic fraction of *Lactobacillus casei* and *Bifidobacterium longum*. *J. Vet. Sci.* **5:** 41–48.
51. Lee, K. H., J. Y. Park, S. J. Jeong, G. H. Kwon, H. J. Lee, H. C. Chang, D. K. Chung, J. H. Lee, and J. H. Kim. 2007. Characterization of paraplantaricin C7, a novel bacteriocin produced by *Lactobacillus paraplantarum* C7 isolated from *kimchi*. *J. Microbiol. Biotechnol.* **17:** 287–296.
52. Lee, K. W. and H. J. Lee. 2006. The roles of polyphenols in cancer chemoprevention. *Biofactors* **26:** 105–121.
53. Lee, K. W., H. J. Lee, H. Y. Cho, and Y. J. Kim. 2005. Role of the conjugated linoleic acid in the prevention of cancer. *Crit. Rev. Food Sci. Nutr.* **45:** 135–144.
54. Lim, B. K., R. Mahendran, Y. K. Lee, and B. H. Bay. 2002. Chemopreventive effect of *Lactobacillus rhamnosus* on growth of a subcutaneously implanted bladder cancer cell line in the mouse. *Jpn. J. Cancer Res.* **93:** 36–41.
55. Lin, D. C. 2003. Probiotics as functional foods. *Nutr. Clin. Pract.* **18:** 497–506.
56. Lin, M. Y. and F. J. Chang. 2000. Antioxidative effect of intestinal bacteria *Bifidobacterium longum* ATCC 15708 and *Lactobacillus acidophilu*s ATCC 4356. *Dig. Dis. Sci.* **45:** 1617–1622.
57. Lin, M. Y. and C. L. Yen. 1999. Antioxidative ability of lactic acid bacteria. *J. Agric. Food Chem.* **47:** 1460–1466.
58. Lin, M. Y. and C. L. Yen. 1999. Inhibition of lipid peroxidation by *Lactobacillus acidophilus* and *Bifidobacterium longum*. *J. Agric. Food Chem.* **47:** 3661–3664.
59. Lorca, G. L., T. Wadstrom, G. F. Valdez, and A. Ljungh. 2001. *Lactobacillus acidophilus* autolysins inhibit *Helicobacter pylori in vitro*. *Curr. Microbiol.* **42:** 39–44.
60. Matsuzaki, T. 1998. Immunomodulation by treatment with *Lactobacillus casei* strain *Shirota*. *Int. J. Food Microbiol.* **41:** 133–140.
61. Nanji, A. A., U. Khettry, and S. M. Sadrzadeh. 1994. *Lactobacillus* feeding reduces endotoxemia and severity of experimental alcoholic liver (disease). *Proc. Soc. Exp. Biol. Med.* **205:** 243–247.
62. Oberfield, R. A., G. Steele Jr., J. L. Gollan, and D. Sherman. 1989. Liver cancer. *CA Cancer J. Clin.* **39:** 206–218.
63. Ohashi, Y., S. Nakai, T. Tsukamoto, N. Masumori, H. Akaza, N. Miyanaga, T. Kitamura, K. Kawabe, T. Kotake, M. Kuroda, S. Naito, H. Koga, Y. Saito, K. Nomata, M. Kitagawa, and Y. Aso. 2002. Habitual intake of lactic acid bacteria and risk reduction of bladder cancer. *Urol. Int.* **68:** 273–280.
64. Orrhage, K., E. Sillerstrom, J. A. Gustafsson, C. E. Nord, and J. Rafter. 1994. Binding of mutagenic heterocyclic amines by intestinal and lactic acid bacteria. *Mutat. Res.* **311:** 239–248.
65. Park, A., A. Rae-Jun, K. H. Lee, S. J. Kim, J. Y. Park, S. J. Nam, H. D. Yun, H. J. Lee, H. C. Chang, D. K. Chung, J. H. Lee, Y. H. Park, and J. H. Kim. 2002. Isolation of *Lactococcus lactis* strain with β-galactosidase activity from *kimchi* and cloning of *lacZ* gene from the isolated strain. *J Microbiol. Biotechnol.* **12:** 157–161.
66. Park, I. S., Y. C. Lee, H. J. Park, T. I. Kim, S. I. Lee, H. Kim, K. S. Chung, and Y. C. Lee-Kim. 2001. *Helicobacter pylori* infection in Korea. *Yonsei Med. J.* **42:** 457–470.
67. Park, S. Y., G. E. Ji, Y. T. Ko, H. K. Jung, Z. Ustunol, and J. J. Pestka. 1999. Potentiation of hydrogen peroxide, nitric oxide, and cytokine production in RAW 264.7 macrophage cells exposed to human and commercial isolates of *Bifidobacterium*. *Int. J. Food Microbiol.* **46:** 231–241.
68. Peek, R. M. Jr. and M. J. Blaser. 2002. *Helicobacter pylori* and gastrointestinal tract adenocarcinomas. *Nat. Rev. Cancer* **2:** 28–37.
69. Perdigon, G., M. E. de Macias, S. Alvarez, G. Oliver, and A. A. de Ruiz Holgado. 1986. Effect of perorally administered lactobacilli on macrophage activation in mice. *Infect. Immun.* **53:** 404–410.
70. Perdigon, G., M. E. Nader de Macias, S. Alvarez, G. Oliver, and A. A. Pesce de Ruiz Holgado. 1987. Enhancement of immune response in mice fed with *Streptococcus thermophilus* and *Lactobacillus acidophilus*. *J. Dairy Sci.* **70:** 919–926.
71. Pool-Zobel, B. L., B. Bertram, M. Knoll, R. Lambertz, C. Neudecker, U. Schillinger, P. Schmezer, and W. H.

Holzapfel. 1993. Antigenotoxic properties of lactic acid bacteria *in vivo* in the gastrointestinal tract of rats. *Nutr. Cancer* **20:** 271–281.
72. Rafter, J., M. Bennett, G. Caderni, Y. Clune, R. Hughes, P. C. Karlsson, A. Klinder, M. O'Riordan, G. C. O'Sullivan, B. Pool-Zobel, G. Rechkemmer, M. Roller, I. Rowland, M. Salvadori, H. Thijs, J. Van Loo, B. Watzl, and J. K. Collins. 2007. Dietary synbiotics reduce cancer risk factors in polypectomized and colon cancer patients. *Am. J. Clin. Nutr.* **85:** 488–496.
73. Reddy, B. S. and A. Rivenson. 1993. Inhibitory effect of *Bifidobacterium longum* on colon, mammary, and liver carcinogenesis induced by 2-amino-3-methylimidazo[4,5-f]quinoline, a food mutagen. *Cancer Res.* **53:** 3914–3918.
74. Rokka, S., A. Pihlanto, H. Korhonen, and V. Joutsjoki. 2006. *In vitro* growth inhibition of *Helicobacter pylori* by lactobacilli belonging to the *Lactobacillus plantarum* group. *Lett. Appl. Microbiol.* **43:** 508–513.
75. 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.
76. Saito, H., H. Tomioka, and K. Nagashima. 1987. Protective and therapeutic efficacy of *Lactobacillus casei* against experimental murine infections due to *Mycobacterium fortuitum* complex. *J. Gen. Microbiol.* **133:** 2843–2851.
77. Sekine, K., T. Kawashima, and Y. Hashimoto. 1994. Comparison of the TNF-α levels induced by human-derived *Bifidobacterium longum* and rat-derived *Bifidobacterium animalis* in mouse peritoneal cells. *Bifidobacteria Microflora* **13:** 79–89.
78. Sellers, W. R. and D. E. Fisher. 1999. Apoptosis and cancer drug targeting. *J. Clin. Invest.* **104:** 1655–1661.
79. Sengupta, N., E. Siddiqui, and F. H. Mumtaz. 2004. Cancers of the bladder. *J. R. Soc. Health* **124:** 228–229.
80. Seow, S. W., J. N. B. Rahmat, A. A. K. Mohamed, R. Mahendran, Y. K. Lee, and B. H. Bay. 2002. *Lactobacillus* species is more cytotoxic to human bladder cancer cells than *Mycobacterium bovis* (*Bacillus Calmette-Guerin*). *J. Urol.* **168:** 2236–2239.
81. Sgouras, D., P. Maragkoudakis, K. Petraki, B. Martinez-Gonzalez, E. Eriotou, S. Michopoulos, G. Kalantzopoulos, E. Tsakalidou, and A. Mentis. 2004. *In vitro* and *in vivo* inhibition of *Helicobacter pylori* by *Lactobacillus casei* strain *Shirota*. *Appl. Environ. Microbiol.* **70:** 518–526.
82. Singh, J., A. Rivenson, M. Tomita, S. Shimamura, N. Ishibashi, and B. S. Reddy. 1997. *Bifidobacterium longum*, a lactic acid-producing intestinal bacterium inhibits colon cancer and modulates the intermediate biomarkers of colon carcinogenesis. *Carcinogenesis* **18:** 833–841.
83. Sporn, M. B. and N. Suh. 2000. Chemoprevention of cancer. *Carcinogenesis* **21:** 525–530.
84. Stiles, M. E. and W. H. Holzapfel. 1997. Lactic acid bacteria of foods and their current taxonomy. *Int. J. Food Microbiol.* **36:** 1–29.
85. Surh, Y. J. 2003. Cancer chemoprevention with dietary phytochemicals. *Nat. Rev. Cancer* **3:** 768–780.
86. Teitelbaum, J. E. and W. A. Walker. 2002. Nutritional impact of pre- and probiotics as protective gastrointestinal organisms. *Annu. Rev. Nutr.* **22:** 107–138.
87. Tolley, D. A., M. K. B. Parmar, K. M. Grigor, and G. Lallemand. 1996. The effect of intravesical mitomycin C on recurrence of newly diagnosed superficial bladder cancer: A further report with 7 years of followup. *J. Urol.* **155:** 1233–1238.
88. van't Veer, P., J. M. Dekker, J. W. Lamers, F. J. Kok, E. G. Schouten, H. A. Brants, F. Sturmans, and R. J. Hermus. 1989. Consumption of fermented milk products and breast cancer: A case-control study in The Netherlands. *Cancer Res.* **49:** 4020–4023.
89. Vanderhoof, J. A. and R. J. Young. 1998. Use of probiotics in childhood gastrointestinal disorders. *J. Pediatr. Gastroenterol. Nutr.* **27:** 323–332.
90. Yasui, H., K. Shida, T. Matsuzaki, and T. Yokokura. 1999. Immunomodulatory function of lactic acid bacteria. *Antonie Van Leeuwenhoek* **76:** 383–389.
91. Yasutake, N., T. Matsuzaki, K. Kimura, S. Hashimoto, T. Yokokura, and Y. Yoshikai. 1999. The role of tumor necrosis factor (TNF)-alpha in the antitumor effect of intrapleural injection of *Lactobacillus casei* strain *Shirota* in mice. *Med. Microbiol. Immunol.* **188:** 9–14.
92. Zhang, X. B. and Y. Ohta. 1991. Binding of mutagens by fractions of the cell wall skeleton of lactic acid bacteria on mutagens. *J. Dairy Sci.* **74:** 1477–1481.