Short communication: Antiproliferative effect of wild *Lactobacillus* strains isolated from fermented foods on HT-29 cells

Y. F. Tuo,*† L. W. Zhang,†¹ H. X. Yi,† Y. C. Zhang,† W. Q. Zhang,‡ X. Han,† M. Du,†
Y. H. Jiao,† and S. M. Wang†

*Department of Food Science and Technology, Gansu Agricultural University, Lanzhou, Gansu 730070, China

†School of Food Science and Engineering, Harbin Institute of Technology, Harbin, Heilongjiang 150090, China

‡Department of Life Science, Tarim University, Alaer, Sinkiang 843300, China

ABSTRACT

In vitro studies, animal models, epidemiology, and human intervention studies provide evidence that some lactic acid bacteria can reduce the risk of certain cancers. In this study, heat-killed bacterial cells, genomic DNA, and cell wall of 7 wild *Lactobacillus* strains isolated from traditional fermented foods in western China were tested in vitro for cytotoxicity on colonic cancer cell line HT-29 by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The heat-killed bacterial cells, genomic DNA, and cell wall of the 7 strains exhibited direct antiproliferative activities against HT-29 cells. Among the strains, the cellular components of *Lactobacillus coryniformis* ssp. *torquens* T3L exerted marked antiproliferative activities against HT-29 cells. The maximum inhibition rates of HT-29 cells by the heat-killed bacterial cells (1×10^7 cfu/mL), cell wall (20 µg of protein/mL) and genomic DNA (100 µg/mL) of *L. coryniformis* ssp. *torquens* T3L were 30, 44.9, and 35.9%, respectively. The results indicate that the heat-killed bacterial cells, cell wall, and genomic DNA of the 7 wild *Lactobacillus* strains could inhibit the growth of HT-29 cells.

Key words: traditional Chinese fermented foods, antiproliferative effect, HT-29 cell, *Lactobacillus*

Cancer is one of the major killer diseases of modern humans, and dietary factors clearly have a major impact on cancer development. An extensive body of literatures addresses possible health benefits associated with the consumption of lactic acid bacteria (LAB). Yogurt and LAB have received much attention as potential cancer-preventing agents in the diet. The results of in vitro assays, animal studies, human studies, epidemiological, and intervention studies have demonstrated that some strains of *Lactobacillus* and their fermented products

could reduce the risks of certain types of cancer and inhibit the growth of certain tumors (Lankaputhra and Shah, 1998; Reddy, 1999; Brady et al., 2000; Burns and Rowland, 2000). Some LAB and their metabolites could inhibit the proliferation of tumor cells by direct or indirect interactions (Reddy and Rivenson, 1993; Hirayama and Rafter, 2000; Rafter, 2003; Commane et al., 2005).

Whole bacteria cells, bacteria cell wall, or other cellular components of LAB, as well as their fermented products, could inhibit the growth of cancer cells (Fichera and Giese 1994; Kim et al., 2002). The antiproliferative activity of LAB against the growth of tumor cells might be because of their cytotoxic and apoptotic effects on colon cancer cells in a dose- and time-dependent manner (Fichera and Giese, 1994; Puertollano et al., 2009; Thirabunyanon et al., 2009).

In this study, the cellular components (i.e., heat-killed whole bacteria cells, cell wall, and bacterial genomic DNA) of 7 wild *Lactobacillus* strains isolated from Chinese traditional fermented foods, such as fermented milk and vegetable juice made locally in western China, were screened and compared for their antiproliferative activities against human colon cancer cell line HT-29. No commercial starter cultures are used in the preparation of these fermented foods. Previous fermented product is normally used as a source of inoculants. The fermentation of the food occurs in the household at ambient temperature. The traditional foods have a long history of safety and are an important part of the local peoples' daily diet. The objectives of this study were to assess the antitumor activities of lactobacilli isolated from the foods and to identify novel probiotic bacteria.

Lactobacillus strains used in this study were isolated from traditional fermented foods in western China and identified by Biolog System (Biolog, Hayward, CA). Detailed description of the *Lactobacillus* strains is shown in Table 1. The strains were cultured in de Man, Rogosa, and Sharpe (MRS) broth at 37°C and stored at 4°C. The strains were subcultured twice at 37°C for 18 h before use.

Received January 9, 2010.

Accepted March 1, 2010.

¹Corresponding author: lanweizhang@yahoo.com.cn

Table 1. *Lactobacillus* strains used in this study

Strain	Species	Origin ¹
SB5L	<i>Lactobacillus rhamnosus</i>	Traditional fermented yaks' milk food; Subei, Gansu Province, China
SB31L	<i>L. rhamnosus</i>	Traditional fermented yaks' milk food; Subei, Gansu Province, China
M5L	<i>Lactobacillus paracasei</i> ssp. <i>paracasei</i>	Traditional koumiss; Yili, Sinkiang, China
J23L	<i>L. paracasei</i> ssp. <i>paracasei</i>	Traditional fermented vegetable juice; Lanzhou, Gansu Province, China
J5L	<i>L. rhamnosus</i>	Traditional fermented vegetable juice; Lanzhou, Gansu Province, China
G15L	<i>L. paracasei</i> ssp. <i>paracasei</i>	Traditional fermented yaks' milk food; Gannan, Gansu Province, China
T3L	<i>Lactobacillus coryniformis</i> ssp. <i>torquens</i>	Traditional yaks' milk cheese; Lhasa, Tibet, China

¹The fermented foods were made by the local people using traditional method under local climate and environmental conditions.

The *Lactobacillus* strains were cultured in MRS broth at 37°C for 18 h to stationary phase, and colony-forming units (cfu/mL) of the strains were determined by pour plating serial dilutions into MRS agar. The bacterial cells were harvested by centrifugation (5,000 × *g*, 10 min at 4°C), washed twice with sterilized water, suspended in sterilized water, adjusted to a final concentration of 2 × 10⁹ cfu/mL, and then heat-killed at 100°C for 15 min (Chuang et al., 2007).

The cell wall material of the strains was prepared according to the method described by Heumann et al. (1994) and Amrouche et al. (2006). Briefly, after 18 h of incubation, bacterial cells from 50-mL strain cultures were harvested by centrifugation (5,000 × *g*, 10 min at 4°C) and washed twice with PBS (pH 7.2). The pelleted cells were suspended in 10 mL of purified water and broken immediately with 3 or 4 volumes of SiO₂ beads (0.05 to 0.1 mm in diameter) in a vortex mixer at maximum speed for 30 min at 4°C. Unbroken bacterial cells and SiO₂ beads were removed from the suspension by low-speed centrifugation at 2,000 × *g* for 15 min at 4°C, and the cell wall material was sedimented by centrifugation at 30,000 × *g* for 30 min at 4°C. Cell wall material was resuspended in 2 mL of purified water. The protein content of the cell wall extracts was determined by using the Lowry method (Lowry et al., 1951).

Genomic DNA of the *Lactobacillus* strains after an 18-h incubation at 37°C was purified using the phenol-chloroform-isoamyl alcohol extraction method (Ghadimi et al., 2008). The concentration and purity of the DNA preparation were determined by measuring absorbance (optical density, OD) at 230, 260, and 280 nm (OD₂₃₀, OD₂₆₀, and OD₂₈₀). Only DNA with an OD_{260/280} ratio >1.8 and OD_{260/230} ratio ≥2 was used.

Human colonic cancer cell line HT-29 was obtained from the Cancer Institute of the Chinese Academy of Medical Science (Beijing, China). The HT-29 cells were cultured in complete RPMI-1640 medium (Thermo Scientific HyClone, Beverly, MA) supplemented with 10% (vol/vol) inactivated (56°C, 30 min) fetal calf serum (Sijiqing Co. Ltd., Hangzhou, China), and a 1% (vol/vol) mixture of penicillin (10,000 IU/mL) and streptomycin (10,000 µg/mL; Solarbio Co. Ltd., Beijing, China) in a

humidified atmosphere of 5% CO₂ and 95% air at 37°C. The HT-29 cells were incubated for 15 d to become fully differentiated. Then, cell viability was assessed by trypan-blue dye (0.2%) in PBS (pH 7.2) and the cell number was determined by hemocytometer. Cultures with ≥80% cell viability were analyzed for subsequent assays.

The HT-29 cells were added into 96-well plates (Corning Inc., Corning, NY) at 100 µL (1 × 10⁴ cells) per well. The heat-killed bacterial cells, cell wall, and genomic DNA of the lactobacilli were diluted in complete RPMI-1640 media and then added into the plates. The final concentrations of heat-killed bacteria cells were 1 × 10⁵, 1 × 10⁶, and 1 × 10⁷ cfu/mL, corresponding to the ratios of bacteria to peripheral blood mononuclear cells of 1:1, 10:1, and 100:1, respectively. The final protein concentrations of cell wall were 10, 20, and 40 µg of protein/mL, and the final concentrations of bacterial genomic DNA were 25, 50, and 100 µg/mL, respectively. Each assay was repeated 6 times. The control wells were without bacterial components. The test and control wells were supplemented with complete RPMI-1640 medium up to 110 µL per well.

The plates were incubated in a humidified atmosphere of 5% CO₂ and 95% air at 37°C for 68 h. Subsequently, 20 µL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, M5655, Sigma-Aldrich Co., St. Louis, MO) at a concentration of 5 mg/mL in PBS (pH 7.4) was added into each well and the plates were incubated for another 4 h. To dissolve the dark formazan crystals, 150 µL of acidification isopropyl alcohol (SDS, 20 g; 1 M HCl, 2 mL; isopropyl alcohol, 10 mL; H₂O, 100 mL) was added into each well and the plates were incubated for another 3 h. The OD was determined on a spectrophotometric microplate reader (Bio-Rad-500, Bio-Rad Laboratories Inc., Hercules, CA) at a test wavelength of 570 nm. Results were presented as the inhibition rate, calculated using the following equation:

$$\text{Inhibition rate} = [1 - (\text{absorbance in test well}) / (\text{absorbance in control well})] \times 100\%.$$

Table 2. Inhibition rate (%) of HT-29 cancer cell line by heat-killed whole bacterial cells of the *Lactobacillus* strains¹

<i>Lactobacillus</i> strain ²	Concentration of heat-killed bacteria (cfu/mL)		
	1×10^5	1×10^6	1×10^7
G15L	$13.1 \pm 1.3^{bc,B}$	$13.5 \pm 1.9^{cd,B}$	$19.4 \pm 2.1^{b,A}$
J23L	$12.4 \pm 0.6^{bc,A}$	$10.6 \pm 1.0^{ef,A}$	$10.4 \pm 0.8^{c,A}$
J5L	$16.0 \pm 1.3^{a,A}$	$14.2 \pm 1.9^{bc,A}$	$8.8 \pm 1.5^{c,B}$
M5L	$14.4 \pm 1.5^{ab,A}$	$11.8 \pm 0.6^{de,AB}$	$10.1 \pm 0.9^{c,B}$
SB31L	$10.7 \pm 0.6^{c,B}$	$11.8 \pm 1.0^{de,B}$	$19.0 \pm 1.0^{b,A}$
SB5L	$4.6 \pm 0.8^{d,B}$	$8.6 \pm 0.6^{f,A}$	$9.3 \pm 1.0^{c,A}$
T3L	$16.3 \pm 3.3^{a,B}$	$26.9 \pm 1.0^{a,A}$	$30.0 \pm 1.1^{a,A}$

^{a-f}Values with different lowercase superscripts in each column are significantly different ($P < 0.05$).

^{A,B}Values with different uppercase superscripts in each row are significantly different ($P < 0.05$).

¹Values are means of triplicate determinations $\pm$ SD.

²Strain information is shown in Table 1.

Antiproliferative assays were performed in triplicate. Statistical analyses were carried out with SPSS14.0 for Windows (SPSS Inc., Chicago, IL). Significant differences between treatments were tested by ANOVA followed by Fisher's least significant difference (LSD) method with a level of significance of $P < 0.05$.

The inhibition rates of HT-29 cells by the heat-killed bacterial cells, cell walls, and genomic DNA of the *Lactobacillus* strains at different concentrations are shown in Tables 2, 3, and 4, respectively. The maximum inhibition rates of HT-29 cells by the heat-killed bacterial cells of the strains G15L, SB31L, SB5L, and T3L were obtained at a concentration of 1×10^7 cfu/mL, whereas the maximal inhibition rates of strains J23L, J5L, and M5L were obtained at a concentration of 1×10^5 cfu/mL. The inhibition rate of HT-29 cells by the heat-killed bacteria of the strain T3L at the concentration of 1×10^7 cfu/mL was 30.0% and significantly higher ($P < 0.05$) than that achieved by other strains.

The maximal inhibition rates of HT-29 cells by the cell walls of the strains J23L, J5L, M5L, and SB31L were obtained at a concentration of 40 μ g of protein/

mL, whereas the maximal inhibition rates of strains G15L and T3L were obtained at 20 μ g of protein/mL. The inhibition rate of HT-29 cells by the cell wall material of strain T3L at 20 μ g of protein/mL was 44.9%, significantly higher ($P < 0.05$) than that achieved by other strains.

The genomic DNA of the strains J23L and SB5L at 25 μ g/mL did not inhibit the proliferation of HT-29 cells significantly. The inhibition rates of HT-29 cells by genomic DNA of strains J5L and M5L at 25, 50, or 100 μ g/mL were not significantly different ($P > 0.05$). The maximum inhibition rate of HT-29 cells by genomic DNA of strain SB31L was obtained at 50 μ g/mL. The inhibition rates of HT-29 cells by genomic DNA of strain T3L increased as the genomic DNA concentration increased. The inhibition rate of HT-29 by the genomic DNA of the strain T3L at 100 μ g/mL was 35.9%, significantly higher ($P < 0.05$) than that achieved by genomic DNA of other strains.

The results of the current study clearly demonstrated that heat-killed bacteria, bacteria cell wall, and bacteria genomic DNA of 7 wild *Lactobacillus* strains inhibited the growth of colonic tumor HT-29 cells.

Table 3. Inhibition rate (%) of HT-29 cancer cell line by cell wall of the *Lactobacillus* strains¹

<i>Lactobacillus</i> strain ²	Concentration of bacterial cell wall (μ g of protein/mL)		
	10	20	40
G15L	$20.4 \pm 0.4^{c,C}$	$30.5 \pm 3.0^{b,A}$	$27.5 \pm 0.6^{b,B}$
J23L	$7.1 \pm 1.0^{d,B}$	$20.5 \pm 0.8^{c,A}$	$21.3 \pm 1.8^{c,A}$
J5L	$24.1 \pm 2.2^{b,B}$	$20.8 \pm 2.4^{c,C}$	$28.3 \pm 1.8^{b,A}$
M5L	$17.1 \pm 2.8^{c,C}$	$21.1 \pm 2.3^{c,B}$	$26.0 \pm 0.4^{b,A}$
SB31L	$10.3 \pm 2.0^{d,B}$	$14.3 \pm 0.8^{d,A}$	$15.2 \pm 0.8^{d,A}$
SB5L	$17.2 \pm 2.6^{c,A}$	$14.3 \pm 1.4^{d,AB}$	$12.1 \pm 1.2^{e,B}$
T3L	$29.7 \pm 1.8^{a,C}$	$44.9 \pm 2.5^{a,A}$	$34.9 \pm 3.1^{a,B}$

^{a-e}Values with different lowercase superscripts in each column are significantly different ($P < 0.05$).

^{A-C}Values with different uppercase superscripts in each row are significantly different ($P < 0.05$).

¹Values are means of triplicate determinations $\pm$ SD.

²Strain information is shown in Table 1.

Table 4. Inhibition rate (%) of HT-29 cancer cell line by genomic DNA of the *Lactobacillus* strains¹

<i>Lactobacillus</i> strain ²	Concentration of genomic DNA (μ g of DNA/mL)		
	25	50	100
G15L	22.2 $\pm$ 1.5 ^{b,A}	17.0 $\pm$ 1.7 ^{c,B}	6.9 $\pm$ 1.3 ^{d,C}
J23L	— ³	4.8 $\pm$ 0.8 ^{e,B}	9.3 $\pm$ 1.4 ^{cd,A}
J5L	26.5 $\pm$ 1.4 ^{a,A}	23.5 $\pm$ 1.4 ^{b,A}	25.9 $\pm$ 1.2 ^{b,A}
M5L	7.2 $\pm$ 0.6 ^{d,A}	7.6 $\pm$ 2.6 ^{e,A}	9.3 $\pm$ 0.8 ^{cd,A}
SB31L	25.0 $\pm$ 0.6 ^{a,B}	30.9 $\pm$ 2.5 ^{a,A}	26.5 $\pm$ 1.4 ^{b,B}
SB5L	—	12.6 $\pm$ 1.4 ^{d,A}	11.5 $\pm$ 3.3 ^{c,A}
T3L	12.4 $\pm$ 2.7 ^{c,C}	30.9 $\pm$ 1.7 ^{a,B}	35.9 $\pm$ 2.5 ^{a,A}

^{a-e}Values with different lowercase superscripts in each column are significantly different ($P < 0.05$).

^{A-C}Values with different uppercase superscripts in each row are significantly different ($P < 0.05$).

¹Values are means of triplicate determinations $\pm$ SD.

²Strain information is shown in Table 1.

³No antiproliferative effect observed.

The antiproliferative activities of the cellular components of the 7 *Lactobacillus* strains against HT-29 cells might be ascribed to the cytotoxic activities of the cellular components. It was reported that cell wall peptidoglycan and whole bacterial cells of some lactobacilli and bifidobacteria had antiproliferative activities against tumor cells because of cytotoxic activity (Sekine et al., 1985; Fichera and Giese, 1994). Moreover, the cytoplasm (Kim et al., 2002) and cytoplasm extract (You et al., 2004) of LAB had significant antiproliferative effects against several cancer cell lines because of their cytotoxicity.

The difference in antiproliferative activities against HT-29 cells among the 7 *Lactobacillus* strains might be correlated to differences in the cellular components of the strains. In gram-positive bacteria, the cell wall consists of several characteristic structures: a thick, multilayered peptidoglycan sacculus decorated with proteins, teichoic acids, and polysaccharides and, in some species, surrounded by an outer shell of proteins packed in a paracrystalline layer. These molecular structures provide the bacteria with species- and strain-specific properties (Lebeer et al., 2008). Seow et al. (2002) showed that the anticancer activities and the mechanism behind the anticancer abilities of *Lactobacillus casei* strain Shirota, *Lactobacillus rhamnosus* strain GG, and *Mycobacterium bovis* against human bladder cancer cell lines were different. Salinas et al. (2008) showed that the cytoplasmic extracts of *Lactobacillus delbrueckii* ssp. *lactis* had stronger antiproliferative effects on SAF-1 and EPC cell lines than that of strain 51M6 (genus *Shewanella*). These results confirmed that the antiproliferative effect on tumor cells exerted by probiotic bacteria was species or strain dependent. The antiproliferative activities against HT-29 cells exerted by the 7 *Lactobacillus* strains used in this study were also different.

In conclusion, all heat-killed bacterial cells, cell wall, and genomic DNA of the 7 wild *Lactobacillus* exerted antiproliferative activities against colonic tumor cell line HT-29. These results indicate the possibility of using the cellular components of the strains as functional ingredients for food applications.

ACKNOWLEDGMENTS

This work was financially supported by National High-Tech R&D Program Grants (2007AA10Z354, 2006BAD04A06, 2006BAD04A09) from the Ministry of Science and Technology of the People's Republic of China, and R&D Program Grants (2004) from Educational Committee of Heilongjiang Province. We also appreciate the valuable advice from Baixiang Li at Harbin Medical University (Harbin, China).

REFERENCES

- Amrouche, T., Y. Boutin, G. Prioult, and I. Fliss. 2006. Effects of bifidobacterial cytoplasm, cell wall and exopolysaccharide on mouse lymphocyte proliferation and cytokine production. *Int. Dairy J.* 16:70–80.
- Brady, L. J., D. D. Gallaher, and F. F. Busta. 2000. The role of probiotic cultures in the prevention of colon cancer. *J. Nutr.* 130:410S–414S.
- Burns, A. J., and I. R. Rowland. 2000. Anti-carcinogenicity of probiotics and prebiotics. *Curr. Issues Intest. Microbiol.* 1:13–24.
- Chuang, L., K. G. Wu, C. Pai, P. S. Hsieh, J. J. Tsai, J. H. Yen, and M. Y. Lin. 2007. Heat-killed cells of lactobacilli skew the immune response toward T helper 1 polarization in mouse splenocytes and dendritic cell-treated T cells. *J. Agric. Food Chem.* 55:11080–11086.
- Commane, D., R. Hughes, C. Shortt, and I. Rowland. 2005. The potential mechanisms involved in the anti-carcinogenic action of probiotics. *Mutat. Res. Fund. Mol. Med.* 591:276–289.
- Fichera, G. A., and G. Giese. 1994. Non-immunologically mediated cytotoxicity of *Lactobacillus casei* and its derivative peptidoglycan against tumor cell lines. *Cancer Lett.* 85:93–103.
- Ghadimi, D., R. Fölster-Holst, M. de Vrese, P. Winkler, K. J. Heller, and J. Schrezenmeier. 2008. Effect of probiotic bacteria and their genomic DNA on TH1/TH2-cytokine production by peripheral

- blood mononuclear cells (PBMCs) of healthy and allergic subjects. *Immunobiology* 213:677–692.
- Heumann, D., C. Barras, A. Severin, M. P. Glauser, and A. Tomasz. 1994. Gram-positive cell walls stimulate synthesis of tumor necrosis factor alpha and interleukin-6 by human monocytes. *Infect. Immun.* 62:2715–2721.
- Hirayama, K., and J. Rafter. 2000. The role of probiotic bacteria in cancer prevention. *Microbes Infect.* 2:681–686.
- 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.
- Lankaputhra, W. E. V., and N. P. Shah. 1998. Antimutagenic properties of probiotic bacteria and of organic acids. *Mutat. Res. Fund. Mol. Med.* 397:169–182.
- Lebeer, S., J. Vanderleyden, and S. C. J. de Keersmaecker. 2008. Genes and molecules of lactobacilli supporting probiotic action. *Microbiol. Mol. Biol. Rev.* 72:728–764.
- Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265–275.
- Puertollano, E., M. A. Puertollano, L. Cruz-Chamorro, G. Á. de Cienfuegos, A. Ruiz-Bravo, and M. A. de Pablo. 2009. Effects of concentrated supernatants recovered from *Lactobacillus plantarum* on *Escherichia coli* growth and on the viability of a human promyelocytic cell line. *J. Appl. Microbiol.* 106:1194–1203.
- Rafter, J. 2003. Probiotics and colon cancer. *Best Pract. Res. Clin. Gastroenterol.* 17:849–859.
- Reddy, B. S. 1999. Possible mechanisms by which pro- and prebiotics influence colon carcinogenesis and tumor growth. *J. Nutr.* 129:1478S–1482S.
- 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.
- Salinas, I., J. Meseguer, and M. A. Esteban. 2008. Antiproliferative effects and apoptosis induction by probiotic cytoplasmic extracts in fish cell lines. *Vet. Microbiol.* 126:287–294.
- Sekine, K., T. Toida, M. Saito, M. Kuboyama, T. Kawashima, and Y. Hashimoto. 1985. A new morphologically characterized cell wall preparation (whole peptidoglycan) from *Bifidobacterium infantis* with a higher efficacy on the regression of an established tumor in mice. *Cancer Res.* 45:1300–1307.
- 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.
- Thirabunyanon, M., P. Boonprasom, and P. Niamsup. 2009. Probiotic potential of lactic acid bacteria isolated from fermented dairy milks on antiproliferation of colon cancer cells. *Biotechnol. Lett.* 31:571–576.
- You, H. J., D. K. Oh, and G. E. Ji. 2004. Anticancerogenic effect of a novel chiroinositol-containing polysaccharide from *Bifidobacterium bifidum* BGN4. *FEMS Microbiol. Lett.* 240:131–136.