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Short communication: Antiproliferative effect of 8 different *Lactobacillus* strains on K562 cells

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ABSTRACT

Some strains of *Lactobacillus* genus have antiproliferative activities against cancer cells. However, until now, the exact effector molecules of *Lactobacillus* strains with anticancer activity have not been identified. The aim of the present study was to explore which fraction of the *Lactobacillus* cells exerts the highest antiproliferative effect. For this purpose, the heat-killed bacterial cells, bacterial cell wall extract, and genomic DNA of 8 *Lactobacillus* strains were prepared to assess their antiproliferative activities against human myeloid leukemia cell lines K562. The heat-killed bacterial cells of the 8 lactobacilli strains exerted antiproliferative effect on K562 cells, and the inhibition rates exerted by the heat-killed bacterial cells of the strains G15AL, M5AL, SB31AL, SB5AL, and T3AL were significantly higher than those exerted by the cell walls and genomic DNA of the strains. The bacterial DNA of G15AL exerted higher antiproliferative effect on K562 cells. The exact effector molecules and the effect mechanism of the strains should be further explored for the application of these strains as probiotic strains or bioactive probiotic molecules.
Key words: *Lactobacillus*, antiproliferative effect, K562 cell

Short Communication

Some indirect proofs based largely on laboratory studies showed that lactic acid bacteria (**LAB**) had anticancer activity. Researchers proved that *Lactobacillus casei* (Tomita et al., 1994), *Lactobacillus rhamnosus* (Goldin et al., 1996), *Lactobacillus acidophilus* (McIntosh et al., 1999), and *Bifidobacterium longum* (Reddy and Rivenson, 1993; Singh et al., 1997) could inhibit the proliferation of tumors in rodent bodies induced by mutagen or by transplantation. Whole bacteria cells, bacteria cell wall, or other cellular components of LAB 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 due to their cytotoxic and apoptotic effects on cancer cells in a dose- and time-dependent manner (Fichera and Giese, 1994; Puertollano et al., 2009; Thirabunyanon et al., 2009).

Our previous study reported that 7 *L. rhamnosus* strains isolated from Chinese traditional fermented foods could inhibit the growth of human colonic cancer cell line HT-29 (Tuo et al., 2010). In the current study, we assessed the antiproliferative effect of the cellular components (i.e., heat-killed whole bacteria cells, cell wall, and bacterial genomic DNA) of 7 *Lactobacillus* strains and another strain from human feces on human myeloid leukemia cell lines K-562.

Information about the 8 *Lactobacillus* strains used in our study is listed 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. The counts (cfu/mL) of the strains were determined by plating serial 10-fold dilution into MRS-agar.

To prepare heat-killed bacterial cells of the *Lactobacillus* strains, bacterial cells from 18-h *Lactobacillus* strain cultures were harvested by centrifugation at 5,000 × *g* for 10 min at 4°C (Micro fuge-18, Low Temperature Superspeed Centrifuge, Beckman Coulter, Brea, CA) and washed twice with PBS buffer (pH 7.2). Heat treatment was carried out according to the method of Lankaputhra and Shah (1998). The cell suspensions in PBS containing 10^9 cfu/mL were heat treated in test tubes (15 × 150 mm) by immersion in a boiling water bath at 100°C for 20 min. After heat treatment, the cells were cooled immediately in ice water and vortexed for about 5 min to break any coagulum formed during heating. Then the heat-killed bacterial cells were resuspended in RPMI-1640 medium (Thermo Scientific HyClone, Thermo Fisher Scientific Inc., Waltham, MA) at a concentration of 2×10^9 cfu/mL. They were then plated in MRS agar to determine the efficacy of heat treatment. The cell wall of the *Lactobacillus* strains was prepared

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Table 1. *Lactobacillus* strains used in this study

Strain	Species	Origin[1]
SB5AL	*Lactobacillus rhamnosus*	Traditional fermented yak milk food; Subei, Gansu Province, China
SB31AL	*L. rhamnosus*	Traditional fermented yak milk food; Subei, Gansu Province, China
M5AL	*Lactobacillus paracasei* ssp. *paracasei*	Traditional koumiss; Yili, Sinkiang
J23ANL	*L. paracasei* ssp. *paracasei*	Traditional fermented vegetable juice; Lanzhou, Gansu Province, China
J5ANL	*L. rhamnosus*	Traditional fermented vegetable juice; Lanzhou, Gansu Province, China
G15AL	*L. paracasei* ssp. *paracasei*	Traditional fermented yak milk food; Gannan, Gansu Province, China
IN1ANL	*L. rhamnosus*	Human feces from healthy infant, Haerbin, Heilongjiang Province, China
T3AL	*Lactobacillus coryniformis* ssp. *torquens*	Traditional yak milk cheese; Lhasa, Tibet

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

according to the method described by Heumann et al. (1994) and Amrouche et al. (2006). Briefly, after 18 h of incubation, the bacteria cells from 50 mL of lactobacilli culture were harvested by centrifugation (5,000 × *g*, 10 min, 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 SiO_2 beads (diameter = 0.05–0.1 mm) in a vortex mixer at maximum speed for 30 min at 4°C. Unbroken bacterial cells and SiO_2 beads were separated from the suspension by low-speed centrifugation at 2,000 × *g* for 15 min at 4°C. The homogenate was recentrifuged at 30,000 × *g* for 30 min at 4°C to pellet cell wall. Cell wall material was resuspended in 2 mL of purified water. The protein content of the cell wall extracts was determined by the Lowry method (Lowry et al., 1951). Then the cell wall was diluted in RPMI-1640 medium. Genomic DNA of the *Lactobacillus* strains incubated for 18 h at 37°C was purified by phenol-chloroform-isoamyl alcohol extraction method (Ghadimi et al., 2008). The concentration and purity of all DNA preparations were determined by measuring absorbance at 230, 260, and 280 nm [optical density $(\mathbf{OD})_{230}$, OD_{260}, and OD_{280}, respectively]. Only the DNA with the $OD_{260/280}$ ratio >1.8 and $OD_{260/230} \geq 2$ was used. The DNA was diluted in RPMI-1640 medium and stored at −20°C.

Experiments to evaluate the antiproliferative effects of the *Lactobacillus* strain cell fractions on human myeloid leukemia cell line K562 were carried out. Human myeloid leukemia cell lines K-562 were obtained from the Cancer Institute of the Chinese Academy of Medical Science (Beijing, China). The K-562 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% CO_2 and 95% air at 37°C. The K-562 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 K-562 cells were added into 96-well plates (Corning Inc., Corning, NY) at 100 μL (1×10^4 cells) per well. The heat-killed bacterial cells, cell wall, and genomic DNA of the lactobacilli were diluted in complete RPMI-1640 medium and then added into the plates. The final concentrations of heat-killed bacteria cells were 1×10^5, 1×10^6, and 1×10^7 cfu/mL, corresponding to the ratios of bacteria to K562 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% CO_2 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 equation

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

Antiproliferative assays were performed in triplicate. Statistical analyses were carried out with SPSS 14.0 for Windows (SPSS Inc., Chicago, IL). Significant differences between treatments were tested by ANOVA