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ORIGINAL ARTICLE

Evaluation of probiotic properties of *Lactobacillus* strains isolated from traditional Chinese cheese

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Abstract Twenty-seven *Lactobacillus* strains isolated from traditional Chinese cheese were evaluated for their probiotic potential by in vitro and in vivo tests. Seven strains were selected as they showed high resistance to low pH and simulated gastrointestinal juice. Further study indicated that five strains exhibited good adhesion to Caco-2 cells. Bile salt hydrolase (BSH) and high β-galactosidase activities were shown by the five strains. They also showed various antimicrobial activity against pathogens. Among the five strains, an atypical resistance to chloramphenicol was detected in strain BJFU 10241. After a series of tests, the *cat* gene was observed in plasmids but not in genomic DNA. In vivo experiments showed that strains BJFU 10256, BJFU 10041, BJFU 10205, and BJFU 10025 could regulate intestinal flora. Significant increases in *Lactobacillus* spp. were observed. Moreover, the numbers of *Enterobacter* and *Clostridium perfringens* decreased significantly. The ability of the four strains to lower serum total and low density lipoprotein cholesterol in mice was observed, and this property was enhanced by ingestion of cell suspension compared to fermented milk. These four strains showed the best probiotic potential and could be used in fermented food in the future.

Keywords Probiotics · *Lactobacillus* · Adhesion · Mice

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Introduction

Probiotics are live micro-organisms that, when administered in adequate amounts, confer a health benefit on the host (FAO/WHO 2001). A variety of lactic acid bacteria (LAB) have been demonstrated to have a beneficial effect and some strains are used as adjunct cultures in various types of food or in adjuvant therapy (Zago et al. 2011). *Lactobacillus* belongs to the group of LAB and exerts a beneficial effect when ingested by humans. Some *Lactobacillus* strains have a long history of safe use and are important starters in the dairy industry (Seppo et al. 2003; Vinderola et al. 2007). Traditional naturally fermented dairy products are an important source of *Lactobacillus*. To date, a great number of *Lactobacillus* isolated from traditional fermented dairy products have been reported and some strains display good ability in overcoming physical and chemical barriers in the gastrointestinal tract, especially acid, proteolytic enzymes and bile stresses (Pinto et al. 2006; Pan et al. 2011; Zago et al. 2011).

As potential probiotics, *Lactobacillus* strains must exhibit the following properties: (1) safety; (2) resistance to gastric and intestinal juices; (3) adherence to the intestinal epithelium; (4) antimicrobial activities against pathogens. In addition, organisms should exert some beneficial effects such as production of β-galactosidase, cholesterol reducing ability, enhanced immune response, and cancer prevention (FAO/WHO 2001; Hyronimus et al. 2000; Park et al. 2002). Another important aspect is antibiotic resistance. The resistance genes can be either encoded chromosomally or plasmid-located. In any case, it is undesired that resistance genes are transferred to pathogenic bacteria (Pinto et al. 2006).

Therefore, the purpose of this study was to characterize potential probiotic *Lactobacillus* strains isolated from traditional Chinese cheese (Xinjiang, China). A series of in vitro and in vivo analyses were performed to evaluate their probiotic properties.

Materials and methods

Strains and cell culture conditions

Twenty-seven *Lactobacillus* strains were isolated from traditional Chinese cheese (Xinjiang, China). The strains were stored at −80 °C in MRS (De Man Rogosa Sharpe) broth, supplemented with 20 % (v/v) glycerol. For routine analysis, strains were sub-cultured twice in MRS broth, at 37 °C for 18 h.

Caco-2 cell was provided by Peking Union Medical College. The Caco-2 cells, derived from human colon adenocarcinoma, were maintained in DMEM (Dulbecco's Modified Eagle Medium), which contained 4.50 g/L glucose, 20 % fetal bovine serum, 0.10 mM nonessential amino acids, 0.05 mg/mL penicillin and 0.1 mg/mL streptomycin. The cell line was incubated at 37 °C with a humidified atmosphere of 5 % CO_2.

Acid tolerance

Lactobacillus strains (10^8 CFU/mL) were added to 10 mL PBS (phosphate-buffered saline, pH 2.5) and mixed. Samples were incubated at 37 °C for 3 h. Viable counts were then determined on MRS agar plates. Survival rates were calculated according to the following formula:

$$\text{Survival rate}\ (\%) = C_1/C_0 \times 100\%$$

where C_1 represents the viable count after treatment by acid and C_0 represents the initial viable count. Counts were expressed in colony forming units (CFU)/mL.

Resistance to simulated gastrointestinal conditions

Simulated gastric and intestinal fluids were prepared according to published reports (Fernandez et al. 2003; Pitino et al. 2010). The cells of *Lactobacillus* strains were harvested at 6,000*g* for 10 min, washed twice with saline, and then inoculated into simulated gastric juice (~10^9 CFU/mL). After the final pH was adjusted to 2.5, the mixtures were incubated anaerobically at 37 °C. Aliquots of this suspension were taken at 0, 1, 2 and 3 h, and viable counts were determined on MRS agar plates. After 3 h, 1 mL suspension was inoculated into 9 mL simulated intestinal fluid (pH 8.0), and incubated at 37 °C anaerobically. Samples were taken at 3, 6, and 12 h and the viable counts in intestinal juice were investigated by a pour plate method using MRS agar.

Bile salt hydrolase activity

For bile salt hydrolase (BSH) testing, fresh cultures were spotted on MRS agar containing 0.5 % sodium salt of taurodeoxycholic acid (Sigma-Aldrich, St. Louis, MO) and 0.3 g/L $CaCl_2$. Colonies with precipitation zones indicated BSH-positive.

β-galactosidase activity

The *o*-nitrophenyl-β-D-galactopyranoside (ONPG) substrate (Sigma) was used to determine β-galactosidase activity according to Pinto et al. (2006). Briefly, 10 mL overnight culture was harvested by centrifugation (6,000 *g* for 10 min at 4 °C) and washed twice with PBS (pH 7.2). After resuspending in the same amount of buffer, the cell suspension was disintegrated by ultrasonic vibrations using a sonifier (Xinzhi, Ningbo, China). Cell extracts were obtained by centrifugation at 12,000 *g* for 15 min at 4 °C and then the β-galactosidase activity was measured. One enzymatic unit was defined as micromoles of ONP liberated from ONPG per milligram of cell extract per minute.

In vitro adhesion assay

For the bacterial adhesion assay, monolayers of Caco-2 cells were prepared in 24-well tissue culture plates. Cells were seeded at densities of 2×10^5 cells/mL. The monolayers were maintained for 14 days and fresh medium was replaced daily. The culture medium was removed and the cells were washed twice with PBS (pH 7.2) before adhesion assay.

Lactobacillus cells were harvested by centrifugation at 6,000*g* for 15 min. The cell pellets were washed twice in the same amount of PBS (pH 7.2) and then suspended in DMEM (containing 10^8 CFU/mL bacteria). Then, 1 mL suspension was added to Caco-2 monolayers prepared as above. After incubation at 37 °C for 1.5 h, the Caco-2 monolayers were washed twice with PBS, and then added to 0.2 mL PBS (containing 1 % Triton X-100). Ten minutes later, 0.8 mL PBS was added and the viable counts were enumerated by a plate method using MRS agar. The adhesion rate was the percentage of viable bacteria compared to the initial amount in the DMEM suspension. *L. acidophilus* NCFM was used as a control.

Antimicrobial activity

A well-diffusion assay was used to evaluate the antimicrobial activity. MRS agar (20 mL) inoculated with 200 μL of indicator strains was poured into plates. Wells (6.00 mm in diameter) were made in the agar. Cell-free supernatants of *Lactobacillus* strains were obtained by centrifuging the culture broth at 8,000*g* for 15 min. The supernatants were filtered through a filter (pore size, 0.22 μm) and adjusted to pH 6.5. Aliquots of 100 μL of supernatants were added into different wells. After incubation at 37 °C for 24 h, the diameters of inhibitory zones were observed.

Determination of antibiotic resistance

Streptomycin, gentamicin, rifampicin, ampicillin, kanamycin, tetracycline and chloramphenicol were used to test for antibiotic resistance. The tests were performed according to Pinto et al. (2006). Strains suspected of carrying acquired resistance were chosen for following tests: (1) a plasmid elimination experiment was performed to investigate whether the resistant gene was located in plasmid DNA (Lou et al. 2002); (2) PCR was employed to detect where the resistant gene was located (plasmid and/or genomic DNA). Plasmid was extracted using a plasmid midi kit (Tiangen, Beijing, China).

Animal studies

A total of 150, 5-week-old Kunming female mice (weight 26–28 g) and standard diet were obtained from Hebei Medical University. The mice were divided randomly into five groups, 15 mice each group. All mice were kept in controlled conditions (temperature, 23±2 °C; humidity, 50±10 %; light, 12/12 h light/dark cycle). The experiments were conducted as follows: all animals were fed with a high fat diet (5.0 g/day for 30 days, consisting of standard diet, 10 % lard and 0.3 % cholesterol). Control groups (Cg-I and Cg-II) received water or 10 % (w/v) sterile skim milk whereas treated groups (Tg-I and Tg-II) received cell suspensions or fermented milk. For preparing cell suspensions, cultures of each strain were harvested at 6,000 *g* for 10 min, washed twice with saline, and then re-suspended in sterile water. Fermented milk was obtained as follows: 10 % (w/v) sterile skim milk, inoculation 4 %, fermentation at 37 °C for 18 h. The cell concentration in all experiments performed was 10^8 CFU/mL.

Effect of Lactobacillus strains on intestinal flora

Intestinal flora including *Bifidobacterium*, *Lactobacillus*, *Enterobacter*, *Enterococcus* and *Clostridium perfringens* in the feces were determined according to Zhao et al. (2008). Feces were collected from Cg-I and Tg-I groups on day 0 and day 30. Serial 10-fold dilutions were prepared in sterile saline, from 10^{-2} to 10^{-8}. Bacteria were detected on the following selective media: LBS for *Lactobacillus*; TPY for *Bifidobacterium*; VRBDA for *Enterobacter*; Pfizer for *Enterococcus* and TSC for *C. perfringens*. The plates with LBS, TPY and TSC agar were incubated anaerobically, and the other plates were cultured aerobically. After incubation at 37 °C for 48 h, the number of colonies was counted.

After 30 days, all mice were decapitated and blood samples were collected. Serum samples were obtained by centrifugation (3,000*g* for 10 min at 4 °C). TC (total cholesterol), HDL (high density lipoprotein cholesterol) and LDL (low density lipoprotein cholesterol) were examined using mensuration reagent kits (Shanghai Institute of Biological Products, China).

After taking blood samples, the liver and spleen were removed aseptically. Tissue homogenates were prepared in 5 mL sterilized saline. MacConkey agar and LAPTg agar were used to examine bacteria.

Statistical analysis

All experiments and analyses were carried out in triplicate and the values represented as mean values. Microsoft Office Excel 2010 and SPSS (17.0) system software were used for data analysis. One-way ANOVA and independent-samples *t*-test were used for statistical analysis.

Results and discussion

Resistance to acid

The acid tolerance profile of 27 *Lactobacillus* strains was strain specific (Table 1); 13 strains showed a survival rate over 70 % after 3 h of incubation at pH 2.5. Thus these 13 strains were studied further. Among the 13 strains, strains BJFU 10256, BJFU 10257 and BJFU 10260 belonged to *L. plantarum*, strain BJFU 10041 belonged to *L. helveticus*, and the remaining 9 strains belonged to *L. pentosus*.

The pH value of the stomach ranges from 1.5 to 4.5 and the ingestion time is about 3 h. So, acid tolerance is a fundamental property for probiotic microorganisms to survive passage through the stomach (Park et al. 2002). Pennacchia et al. (2004) selected 20 resistant strains from 77 LAB strains that could survive at pH 2.5 for 3 h and showed a survival rate more than 80 %. In this work, acid conditions (pH 2.5 for 3 h) were used as preliminary method for screening probiotic *Lactobacillus* strains.

Resistance to simulated gastric and intestinal fluids

As shown in Table 2, all 13 strains showed high resistance to simulated gastric juice. All strains retained their viability after 3 h, and the viable counts showed decreases of less than 1.5 log cycle with respect to the initial cell concentrations. However, after 12 h exposure to simulated intestinal juice, only seven strains displayed good survival capacity, because cell populations remained over 4 log CFU/mL. Therefore these seven strains were selected for further studies. Among them, strain BJFU 10205 showed the highest level of survival with viable counts of 6.08 log CFU/mL. For strains BJFU 10256 and BJFU 10260, the values were only 4.81 and 4.73 log CFU/mL, respectively.

Table 1 Acid tolerance of different *Lactobacillus* strains

Strain	Survival rate (%)	Strain	Survival rate (%)
Lactobacillus plantarum BJFU 10255	31.24±1.92	*L. pentosus* BJFU 10202	77.26±1.25
L. plantarum BJFU 10256	71.63±1.22	*L. pentosus* BJFU 10204	64.13±2.32
L. plantarum BJFU 10257	76.75±1.54	*L. pentosus* BJFU 10205	82.53±2.13
L. plantarum BJFU 10258	50.67±2.14	*L. pentosus* BJFU 10207	40.66±1.27
L. plantarum BJFU 10259	52.18±2.16	*L. pentosus* BJFU 10208	76.43±2.27
L. plantarum BJFU 10260	80.15±1.66	*L. pentosus* BJFU 10209	65.16±1.24
Lactobacillus helveticus BJFU 10023	63.51±1.73	*L. pentosus* BJFU 10212	74.85±1.54
L. helveticus BJFU 10040	50.84±0.78	*L. pentosus* BJFU 10214	59.87±2.94
L. helveticus BJFU 10041	72.06±1.31	*L. pentosus* BJFU 10215	63.85±1.33
Lactobacillus pentosus BJFU 10151	45.32±2.52	*L. pentosus* BJFU 10218	81.06±1.15
L. pentosus BJFU 10155	84.56±1.03	*L. pentosus* BJFU 10223	51.52±2.36
L. pentosus BJFU 10241	77.46±2.18	*L. pentosus* BJFU 10024	78.17±1.77
L. pentosus BJFU 10199	21.62±1.39	*L. pentosus* BJFU 10025	76.71±1.94
L. pentosus BJFU 10201	62.65±1.87		

A candidate probiotic must be able to survive passage through the gastrointestinal tract and reach the colon environment in adequate numbers (Fernandez et al. 2003). This is an important step towards the selection of probiotics. In this study, all strains exhibited good adaptation to simulated gastric juice. Some recognized commercial probiotics are usually used as controls when evaluating gastrointestinal tolerance. Conway et al. (1987) observed the survival of *L. acidophilus* NCFM in simulated gastric juice. After 3 h, a 2.3 log cycle drop in viable counts occurred. Compared with *L. acidophilus* NCFM, all 13 strains tested here displayed higher survival during gastric digestion. In contrast to gastric juice, some strains selected in this study were sensitive to intestine juice.

Table 2 Gastric and intestinal fluids tolerance of different strains (log CFU/mL)

Strain	Initial	Gastric juice			Intestinal juice		
		1 h	2 h	3 h	3 h	6 h	12 h
BJFU 10256	8.92	8.79	8.41	7.88	7.12	6.72	4.81
BJFU 10257	9.11	8.84	8.66	8.13	5.21	–[a]	–
BJFU 10260	8.87	8.63	8.02	7.94	7.61	7.08	4.73
BJFU 10041	9.05	8.56	8.46	8.17	7.37	6.85	5.81
BJFU 10155	8.95	8.97	8.10	7.68	–	–	–
BJFU 10241	8.94	8.96	8.70	8.13	7.03	6.61	5.53
BJFU 10202	9.08	8.71	8.67	7.66	4.42	–	–
BJFU 10205	8.97	8.91	8.78	8.26	6.83	6.58	6.08
BJFU 10208	9.05	9.02	8.17	7.83	6.27	5.12	–
BJFU 10212	9.07	8.73	8.41	8.19	5.85	4.61	–
BJFU 10218	8.95	8.71	8.24	7.88	5.76	4.18	–
BJFU 10024	9.08	8.84	8.38	8.23	7.84	6.87	5.45
BJFU 10025	9.02	8.65	8.20	8.04	7.69	7.21	5.37

[a] No growth or less than 4.00

Only seven strains showed higher survival even after 12 h of incubation in simulated intestinal fluid. This result was similar to that of a previous study by Mishra and Prasad (2005).

BSH and β-galactosidase activity

As shown in Table 3, all seven strains exhibited BSH activity. All strains showed β-galactosidase activity. The highest activity (9.17 μmol mL^{-1} min^{-1}) was obtained from strain BJFU 10241.

Adhesion properties

As shown in Table 3, all *Lactobacillus* strains tested were able to adhere to Caco-2 cells. Adhesion rates ranged from 2.85 to 9.45 %. Strains BJFU 10041 and BJFU 10241showed stronger adhesion ability (9.45 and 9.12 %, respectively), which were higher than the control strain NCFM ($P<0.05$). However, strains BJFU 10260 and BJFU 10024 exhibited weaker adhesion ability compared with strain NCFM. So these two strains were not further considered in following studies. The values of strains BJFU 10256, BJFU 10205 and BJFU 10025 were 8.43, 7.87 and 8.51 %, respectively.

The ability of probiotic bacteria to adhere to the intestinal epithelium is considered an essential property for beneficial (probiotic) effects (Collado et al. 2005). Adhesion of probiotics has been claimed to be important for competitive inhibition of pathogens and immunoregulation of the host (Pitino et al. 2010). A Caco-2 cell line was used to evaluate the adhesion abilities of *Lactobacillus* strains, as Caco-2 cells exhibit various enterocytic characteristics in vitro (Fogh et al. 1977). In this study, five strains displayed high adhesion capacity to Caco-2 cells compared with the well-known commercial probiotics.

Table 3 Bile salt hydrolase (BSH), β-galactosidase activity and adhesion properties of *Lactobacillus* strains

Strain	BSH	β-Galactosidase activity (μmol mL^{-1} min^{-1})	Adhesion rate (%)
L. plantarum BJFU 10256	+	5.92	8.43
L. plantarum BJFU 10260	+	6.33	2.85
L. helveticus BJFU 10041	+	7.45	9.45
L. pentosus BJFU 10241	+	9.17	9.12
L. pentosus BJFU 10205	+	8.22	7.87
L. pentosus BJFU 10024	+	6.58	4.32
L. pentosus BJFU 10025	+	7.46	8.51
L. acidophilus NCFM			8.83

Antimicrobial activity

The ability of *Lactobacillus* strains to inhibit common pathogens is shown in Table 4. None of the strains tested was able to inhibit *Bacillus subtilis* ATCC 6633. Strain BJFU10256 showed antibacterial activity against both gram negative and gram positive bacteria. Strain BJFU10256 has been demonstrated to produce a bacteriocin (data not shown). Strain BJFU10041 displayed antibacterial ability to five indicator strains. Strains BJFU10241 and BJFU10025 exhibited the same antibacterial spectrum. In contrast, strain BJFU10205 expressed inhibitory activity only against *Staphylococcus aureus*.

Lactobacillus strains can produce one or more antimicrobial active metabolites such as organic acids (mainly lactic and acetic acid), hydrogen peroxide, phenyllactic acid, fatty acids, bacteriocin and antifungal peptides (Reis et al. 2012). Of these metabolites, many studies have focused on bacteriocin and a lot of bacteriocins have been reported (Reis et al. 2012). In present work, five strains showed different antibacterial activities against the indicator strains. Strain BJFU 10256 had been proved to produce a bacteriocin (data not shown). The inhibitory mechanisms of the active strains need further study.

Antibiotic resistance

All five strains were susceptible to streptomycin, gentamicin, rifampicin, ampicillin, kanamycin and tetracycline. Strain BJFU 10241 was resistant to chloramphenicol. Two different plasmids were found in cells of strain BJFU 10241 (data not shown). After elimination of the plasmids, strain BJFU 10241 showed susceptibility to chloramphenicol. After PCR amplification, the *cat* gene was detected in plasmid DNA but not in genomic DNA (data not shown). This indicated that resistance gene was plasmid-located.

Probiotic strains must be safe for human consumption. This safety includes lack of acquired antibiotic resistance and the inability to transfer antibiotic resistance genes. Antibiotic resistance genes are usually located on plasmids. If such plasmids are transferred to other bacteria by conjugation, it may result in highly antibiotic resistant pathogenic bacteria (Cebeci and Gurakan 2003). Danielsen and Wind (2003) reported that all investigated *Lactobacillus* strains displayed resistance to aminoglycosides and ciprofloxacin. Pinto et al. (2006) found that all selected strains were resistant to streptomycin, gentamicin, and ciprofloxacin but that they were susceptible to erythromycin, ampicillin, penicillin, tetracycline and chloramphenicol. In this work, strain BJFU 10241 was resistant only to chloramphenicol but susceptible to the other six antibiotics. So, all five strains were considered safe in this respect.

In vivo trials

Effects on intestinal flora

The changes in fecal bacteria flora are shown in Fig. 1. After ingestion of cell suspensions of each strain, the *Lactobacillus* counts in the gut increased significantly compared with both control group and pre-treatment of each sample (both $P<0.05$). For *Bifidobacterium*, the counts increased

Table 4 Antimicrobial activities of *Lactobacillus* strains

Indicator strains[a]	Diameter of inhibition zone (mm)[b]				
	BJFU 10256	BJFU 10041	BJFU 10241	BJFU 10205	BJFU 10025
Bacillus subtilis ATCC 6633	–	–	–	–	–
Enterococcus faecium CICC 22264	+	+	–	–	–
Enterococcus faecalis CICC 23215	++	+	+	–	++
Staphylococcus aureus ATCC 6538	+++	++	++	+	+++
Escherichia coli ATCC 8739	++	++	+	–	++
Flavobacterium odoratum CICC 23245	+	–	++	–	++
Pseudomonas fluorescens CICC 23250	++	++	–	–	–

[a] *ATCC* American type culture collection; *CICC* China center of industrial culture collection

[b] – No inhibition, + 0–5 mm, ++ 5–10 mm, +++ >10 mm

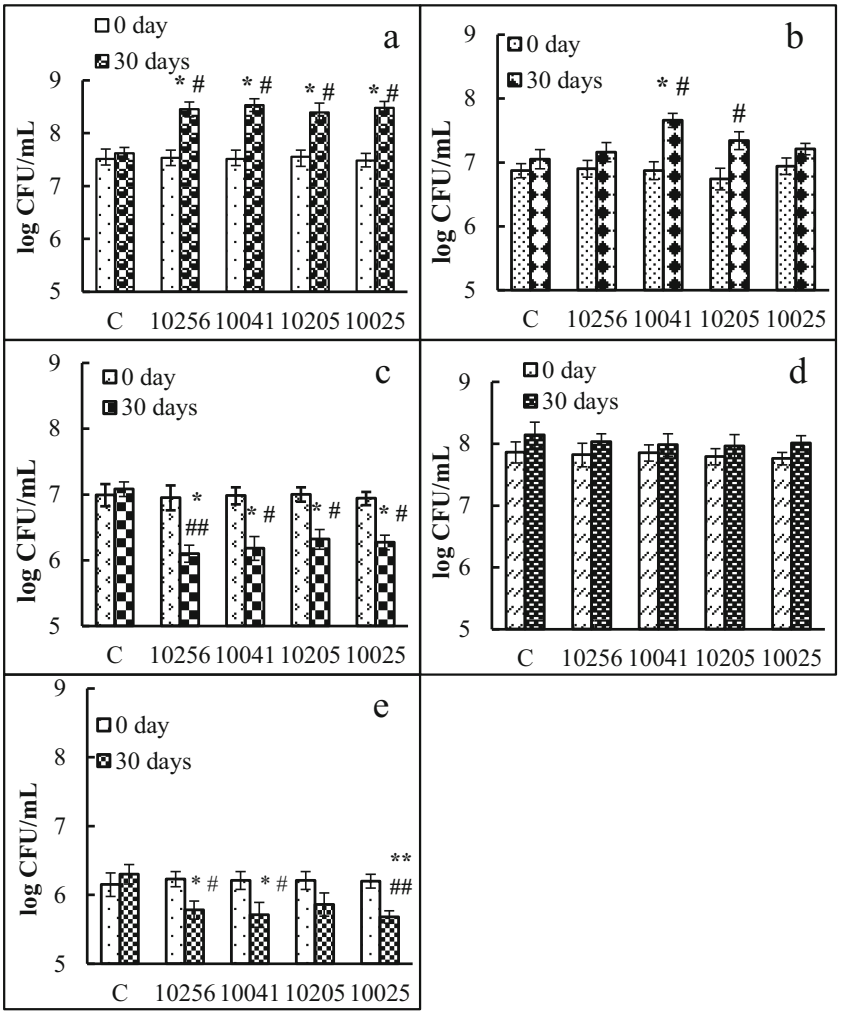

Fig. 1a–e Effects of *Lactobacillus* BJFU strains on intestinal flora of mice. Effects on **a** *Lactobacillus* spp., **b** *Bifidobacterium*, **c** *Enterobacter*, **d** *Enterococcus* and **e** *Clostridium perfringens*

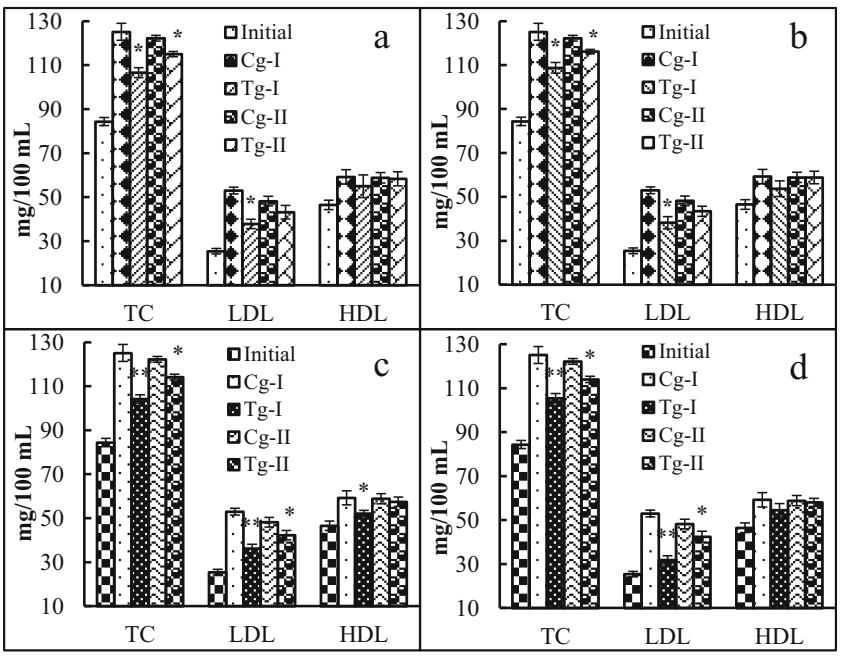

Fig. 2a–d Effects of *Lactobacillus* strains on TC (total cholesterol), HDL (high density lipoprotein cholesterol) and LDL (low density lipoprotein cholesterol) of mice. Strains: **a** BJFU 10256, **b** BJFU 10042, **c** BJFU 10205, **d** BJFU 10025

significantly after ingestion of strain BJFU 10041 (both $P<0.05$) and a significant increase was obtained by strain BJFU 10205 compared to the pre-treatment sample ($P<0.05$), whereas there was no difference for the other strains (both $P>0.05$) (Fig. 1b). The numbers of *Enterobacter* were significantly lower after ingestion of each strain (both $P<0.05$). However, there was no difference in the number of *Enterococcus* (both $P>0.05$). As shown in Fig. 1e, the numbers of *Clostridium perfringens* decreased significantly after ingestion of strains BJFU 10256, BJFU 10041 and BJFU 10025 (both $P<0.05$), especially BJFU 10041 (both $P<0.01$). In contrast, no difference was observed with strain BJFU 10205 (both $P>0.05$).

The intestinal flora is a complex ecosystem and contains a large variety of bacteria. Probiotic strains can influence intestinal flora and promote the health of the host. Some reports indicate that ingestion of LAB can increase the *Lactobacillus* spp. population in the intestinal flora and decrease the populations of *Enterobacter* and *C. perfringens* (Sreekumar and Hosono 2000; Angelakis et al. 2012). In the present study, all tested strains, especially strain BJFU 10041, displayed a beneficial effect in stabilizing the balance of intestinal flora. They induced an increase in beneficial bacteria and a reduction in pathogenic bacteria.

Effects on serum cholesterol levels

Compared with initial values, the levels of TC and LDL increased significantly after 30 days of a high fat diet ($P<0.05$); HDL levels were also higher but the increase was not statistically significant ($P>0.05$). This indicated that the mouse model was successfully established. For strain BJFU 10256, serum TC levels in the treated group (Tg-I and Tg-II) showed a significant decrease ($P<0.05$) compared with control groups (Fig. 2a). This revealed that ingestion of cell suspension or fermented milk could lower the TC level in vivo. The level of LDL decreased significantly after ingestion of cell suspension ($P<0.05$); whereas no significant difference was observed upon treatment with fermented milk ($P>0.05$) (Fig. 2a). Similar results were obtained with strain BJFU 10041 (Fig. 2b). Strains BJFU 10205 and BJFU 10025 were much better in regulating TC and LDL levels (Fig. 2c, d). The TC and LDL levels treated with cell suspensions exhibited a significant decrease ($P<0.01$) for both strains, and the values for fermented milk were also lowered ($P<0.05$). However, none of the tested strains, except strain BJFU 10205, had any effect on regulating HDL level.

The reduction of TC or LDL is considered to lower the risk of cardiovascular disease and various studies have demonstrated that some *Lactobacillus* strains could lower TC and LDL (Adebawo et al. 2008; Pan et al. 2011). The results of the present work suggest that ingestion of cell suspensions or fermented milk of some *Lactobacillus* strains could reduce serum TC and LDL levels in mice. To some extent, the effects of cell suspensions were better than fermented milk. All four strains tested exhibited good capacity in regulating TC and LDL levels in vivo.

Bacterial translocation

No growth of bacterial colonies was detected on either MacConkey agar or LAPTg agar. This suggested that no microbial translocation occurred in the present study. Bacterial translocation is a potential indicator of probiotic toxicity. As a probiotic, the risk of bacterial translocation should be evaluated (Steffen and Berg 1983). In this work, strains did not translocate to the liver or spleen. Therefore, the strains were safe.

Conclusions

In summary, a series of in vitro and in vivo analyses were used to evaluate the probiotic properties of all 27 strains of *Lactobacillus* isolated from traditional Chinese cheese. Finally, four strains (BJFU 10256, BJFU 10041, BJFU 10205, and BJFU 10025) exhibited best probiotic properties, safety characteristics and beneficial effects on host health. These four strains may be used as starter cultures in fermented foods in the future. Further studies are needed to evaluate their technological traits such as growth and survival in food, as well as during production and storage.

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Conflict of interest None.

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