Fermentation characteristics and transit tolerance of probiotic *Lactobacillus casei* Zhang in soymilk and bovine milk during storage

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ABSTRACT

Lactobacillus casei Zhang is a novel strain that was screened out of koumiss collected in Inner Mongolia, and our previous research showed that *L. casei* Zhang has health benefits such as cholesterol-reducing and immunomodulating effects. The fermentation characteristics of *L. casei* Zhang in soymilk and bovine milk and the transit tolerance of *L. casei* Zhang in fermented milk products during refrigerated storage for 28 d were assessed. A faster decrease in pH and faster growth of *L. casei* Zhang during fermentation were observed in soymilk compared with bovine milk at various inoculation rates, probably because of the low pH buffering capacity of soymilk. The fermented bovine milk samples had much higher final titratable acidity (TA) values (between 0.80 and 0.93%) than the soymilk samples (between 0.40 and 0.46%). Dramatic increases in TA values in the fermented soymilk samples during storage were observed, and the TA values of the fermented soymilk samples changed from <0.56% to values between 0.86 and 0.98%. On the other hand, only slight increases in TA were observed in the bovine milk samples during the 28 d of storage. The survival rates of freshly prepared cultures of *L. casei* Zhang in simulated gastric juice at pH 2.0 and 2.5 were 31 and 69%, respectively, and the delivery of *L. casei* Zhang through fermented soymilk and bovine milk significantly improved the viability of *L. casei* Zhang in simulated gastric transit. *Lactobacillus casei* Zhang showed good tolerance to simulated gastric juice and intestinal juice in the fermented soymilk and bovine milk samples, and maintained high viability ($>10^8$ cfu/g) during storage at 4°C for 28 d. Our results indicated that both soymilk and bovine milk could serve as vehicles for delivery of probiotic *L. casei* Zhang, and further research is needed to elucidate the mechanism of the change in pH and TA of *L. casei* Zhang in fermented milk samples during

fermentation and storage and to understand the difference between soy- and milk-based systems.

Key words: *Lactobacillus casei*, soymilk, bovine milk, fermentation characteristic

INTRODUCTION

Koumiss is a traditional fermented alcoholic beverage prepared from mare's milk by the people of the Central Asia steppes, including the Turks, Bashkirs, Kazakhs, Mongols, Yakuts, and Uzbeks, and has been reported to have therapeutic effects in hepatitis, chronic ulcer, and tuberculosis (Park et al., 2006). *Lactobacillus casei* Zhang was a novel strain screened from koumiss collected from Inner Mongolia, and our research has shown that *L. casei* Zhang has health benefits such as cholesterol-reducing and immunomodulating effects (Tuoya et al., 2006) as well as antagonism to *Escherichia coli* in mice (Zhang et al., 2007). In vitro tests indicated that *L. casei* Zhang showed high tolerance to simulated gastric juice, intestine juice, and bile salts, and it exhibited higher viable counts during storage in fermented bovine milk than commercial probiotics such as *Lactobacillus acidophilus* NCFM, *Lactobacillus rhamnosus* GG, *Lactobacillus casei* Shirota, and *Bifidobacterium animalis* Bb12 (our unpublished data) at the same inoculation rate.

According to the Food and Agricultural Organisation (FAO), probiotic bacteria are defined as “live microorganisms that when administered in adequate amounts confer a health benefit on the host” (FAO, 2001). Therefore, in the development of probiotic foods, research is required to select the right vehicle for the delivery of the probiotics to ensure that probiotics are viable throughout the shelf life and overcome the physical and chemical barriers in the gastrointestinal (GI) tract (Samona and Robinson, 1991; Vinderola et al., 2000; Piano et al., 2006). Although yogurt produced from bovine milk is the most popular yogurt in the world, the demand for alternatives to bovine milk products is growing because of bovine milk allergenicity and the unique health benefits provided by other products such as soy products. Soy-based foods could provide unique

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health benefits to the consumers because of their hypolipidemic, anticholesterolemic, and antiatherogenic properties; they could also reduce the risk of hormone-associated health disorders (Favaro Trindade et al., 2001). Therefore, it would be desirable to evaluate how probiotic bacteria perform in various fermented milk products during fermentation and storage.

The objective of the present study was to investigate the fermentation characteristics and transit tolerance of *L. casei* Zhang in soymilk and bovine milk during refrigerated storage and to investigate the possibility of using fermented milk from various sources as vehicles for delivery of probiotic *L. casei* Zhang.

MATERIALS AND METHODS

Bacterial Strains and Reagents

Lactobacillus casei Zhang was isolated from koumiss collected in Xilin Guole of Inner Mongolia (Menghe et al., 2004), and a direct vat-set culture containing 2×10^{11} cfu/g of *L. casei* Zhang was prepared in our laboratory. Nonfat soymilk powder and nonfat dry bovine milk powder were purchased from Wandefu Food Co. Ltd. (Shandong, China) and NZMP Ltd. (Wellington, New Zealand), respectively. Nonfat soymilk powder contained 55 g of protein and 34 g of carbohydrate per 100 g, and nonfat bovine milk powder contained 33 g of protein and 51 g of lactose per 100 g.

Fermented Milk Manufacture

Nonfat soymilk powder or nonfat bovine milk powder was blended with water at 50°C to a total solids content of 6.0 and 11.0 g/100 g, respectively. Each of the hydrated solutions was supplemented with 1.5 g/100 g of glucose, and heated at 85°C for 30 min. Subsequently, the samples were cooled to the incubation temperature (37°C), inoculated with 2×10^6 , 5×10^6 , 1×10^7 , or 2×10^7 cfu/g of *L. casei* Zhang. Fermentation was carried out until the samples with the highest inoculation rate (2×10^7 cfu/g) achieved a pH of 4.5. Then, the samples were cooled to 4°C and stored for 28 d. The pH value, titratable acidity (TA), and viable counts were determined at 0, 6, 12, 16, and 18 h during fermentation and at 0, 7, 14, 21, and 28 d during refrigerated storage. The transit tolerance was analyzed at 0, 1, 14, and 28 d during refrigerated storage. All analyses were performed in triplicate.

Fermentation Characteristics

The pH value was measured using a pHSJ-3F pH meter (Leici, Shanghai, China) with a combined glass

Table 1. Changes in pH and titratable acidity (TA) values in fermented soymilk and bovine milk samples inoculated with *Lactobacillus casei* Zhang during fermentation at 37°C¹

Inoculation level, cfu/g	0 h			6 h			12 h			16 h			18 h		
	pH	TA (%)		pH	TA (%)		pH	TA (%)		pH	TA (%)		pH	TA (%)	
2×10^6															
Soy	6.47 ± 0.00 ^b	0.15 ± 0.00 ^b		6.42 ± 0.01 ^b	0.15 ± 0.01 ^b		ND ²	ND		4.67 ± 0.02	0.41 ± 0.00		ND	ND	
Bovine	6.50 ± 0.00 ^a	0.17 ± 0.00 ^a		6.49 ± 0.01 ^a	0.18 ± 0.00 ^a		6.18 ± 0.04	0.24 ± 0.00		ND	ND		4.63 ± 0.03	0.81 ± 0.03	
5×10^6															
Soy	6.47 ± 0.00 ^b	0.15 ± 0.00 ^b		6.25 ± 0.01 ^b	0.15 ± 0.01 ^b		ND	ND		4.28 ± 0.02	0.46 ± 0.01		ND	ND	
Bovine	6.50 ± 0.00 ^a	0.17 ± 0.00 ^a		6.44 ± 0.01 ^a	0.18 ± 0.01 ^a		6.04 ± 0.04	0.32 ± 0.02		ND	ND		4.51 ± 0.06	0.80 ± 0.00	
1×10^7															
Soy	6.47 ± 0.00 ^b	0.15 ± 0.00 ^b		6.07 ± 0.01 ^b	0.17 ± 0.00 ^b		ND	ND		4.52 ± 0.03	0.40 ± 0.05		ND	ND	
Bovine	6.50 ± 0.00 ^a	0.17 ± 0.00 ^a		6.38 ± 0.01 ^a	0.19 ± 0.00 ^a		5.46 ± 0.10	0.50 ± 0.05		ND	ND		4.54 ± 0.01	0.86 ± 0.09	
2×10^7															
Soy	6.47 ± 0.00 ^b	0.15 ± 0.00 ^b		5.79 ± 0.00 ^b	0.21 ± 0.01 ^a		ND	ND		4.45 ± 0.01	0.43 ± 0.01		ND	ND	
Bovine	6.50 ± 0.00 ^a	0.17 ± 0.00 ^a		6.29 ± 0.00 ^a	0.22 ± 0.00 ^a		5.30 ± 0.01	0.54 ± 0.00		ND	ND		4.50 ± 0.01	0.93 ± 0.00	

^{a,b}Means within a column and inoculation level with different superscript letters are significantly ($P < 0.05$) different.

¹Data are means of triplicate determinations ± standard deviation.

²ND = not determined.

Table 2. Changes in the viable counts of *Lactobacillus casei* Zhang in the fermented soymilk and bovine milk samples during fermentation at 37°C¹

Inoculation level, cfu/g	Viable count				
	0 h	6 h	12 h	16 h	18 h
2 × 10 ⁶					
Soy	6.40 ± 0.05 ^a	6.80 ± 0.11 ^a	ND ²	9.24 ± 0.02	ND
Bovine	6.03 ± 0.10 ^b	6.58 ± 0.04 ^b	7.71 ± 0.17	ND	8.38 ± 0.20
5 × 10 ⁶					
Soy	6.80 ± 0.07 ^a	7.18 ± 0.11 ^a	ND	9.01 ± 0.04	ND
Bovine	6.57 ± 0.08 ^b	7.31 ± 0.07 ^a	8.05 ± 0.04	ND	8.13 ± 0.48
1 × 10 ⁷					
Soy	7.15 ± 0.05 ^a	7.70 ± 0.01 ^a	ND	9.03 ± 0.03	ND
Bovine	7.10 ± 0.11 ^a	7.29 ± 0.34 ^b	8.26 ± 0.14	ND	8.24 ± 0.09
2 × 10 ⁷					
Soy	7.38 ± 0.09 ^a	8.03 ± 0.04 ^a	ND	9.02 ± 0.04	ND
Bovine	7.18 ± 0.48 ^a	7.95 ± 0.01 ^b	8.42 ± 0.20	ND	8.65 ± 0.02

^{a,b}Means within a column and inoculation level with different superscript letters are significantly ($P < 0.05$) different.

¹Data are means of triplicate determinations ± standard deviation.

²ND = not determined.

electrode and temperature probe. Titratable acidity (% lactic acid) was determined by titration with NaOH using phenolphthalein as the indicator (National Standards of the People's Republic of China, 1996). The viable counts of *L. casei* Zhang were enumerated according to the description of Tharmaraj and Shah (2003).

Transit Tolerance

Preparation of Simulated Gastrointestinal Juices. Phosphate buffered saline was prepared with 0.8 g of NaCl, 0.02 g of KH₂PO₄, and 0.115 g of Na₂HPO₄/100 g (Makras et al., 2006). The pH of PBS was adjusted to 2.0, 2.5, or 8.0, respectively, and PBS was sterilized by autoclaving at 121°C for 15 min. The simulated gastric juice was prepared by supplementing PBS (pH 2.0 and 2.5) with pepsin (1:10,000, Sigma Chemical Co., St. Louis, MO). Simulated intestinal juice was prepared by supplementing PBS (pH 8.0) with trypsin (1:250, Sigma Chemical Co.).

Transit Tolerance of *L. casei* Zhang. *Lactobacillus casei* Zhang was grown in de Man, Rogosa, and Sharpe (MRS) broth at 37°C for 18 h and centrifuged at 2,500 × *g* at 4°C for 10 min. The collected cells were resuspended in sterile saline and inoculated into the simulated gastric juice (pH 2.0 or 2.5) at 10⁸ cfu/mL. Because the pH in the human stomach ranges from 1 (during fasting) to 4.5 (after a meal) and food ingestion can take up to 3 h, tolerance was assayed by determining the total viable count of *L. casei* Zhang after a 3-h incubation in simulated gastric juice (pH 2.0 or 2.5). Then, 1.0 mL of the solution was taken and added to 9.0 mL of simulated intestinal juice (pH 8.0) and

incubated at 37°C for 8 h. The small intestine transit tolerance of *L. casei* Zhang was assayed by determining the total viable count after incubation for 4 and 8 h.

Transit Tolerance of *L. casei* Zhang in the Fermented Soymilk and Bovine Milk Samples. One gram of the fermented soymilk or bovine milk sample was mixed with 9.0 mL of simulated gastric juice (pH 2.0 or 2.5) and incubated at 37°C for 3 h. Then, 1.0 mL of the solution was taken and added to 9.0 mL of simulated intestinal juice (pH 8.0), and the sample was incubated at 37°C for 8 h for the transit tolerance analysis. During the process, the resistance of *L. casei* Zhang to simulated gastric juice was analyzed after 3 h of incubation in simulated gastric juice, and transit tolerance was analyzed after 4- and 8-h incubations in simulated intestinal juice as described above.

Determination of Total Viable Count and Survival Rate

Total viable counts of *L. casei* Zhang were determined by a pour-plate method using MRS agar after serial dilution in maximum recovery. The MRS agar plates were incubated anaerobically at 37°C for 48 h, and survival rate was calculated according to the following equation:

$$\text{survival rate}(\%) = \frac{\log \text{cfu } N_1}{\log \text{cfu } N_0} \times 100\%,$$

where N_1 = the total viable count of *L. casei* Zhang after treatment by simulated gastrointestinal juices, and N_0 = the total viable count of *L. casei* Zhang before treatment.

Statistical Analysis

Data were analyzed by ANOVA using Proc Mixed (SAS Institute Inc., Cary NC). Significant differences between means were determined using Fisher's protected least significant difference test. Significant differences were determined at $\alpha = 0.05$. All analyses were conducted in triplicate.

RESULTS AND DISCUSSION

Fermentation Characteristics and Viability of *L. casei* Zhang During Fermentation

pH and TA. The changes in pH and TA of the soymilk and bovine milk samples inoculated with 4 levels of *L. casei* Zhang are shown in Table 1. During fermentation, gradual decreases in pH and increases in TA were observed in both the fermented soymilk and bovine milk samples, and the higher inoculation levels generally resulted in higher acidification rates. A faster drop in pH during fermentation was observed in the soymilk samples compared with the bovine milk samples at all inoculation rates. The pH values of the soymilk and bovine milk samples generally reached 4.5 within 16 and 18 h of fermentation, respectively. The fermented bovine milk samples had much higher final TA values (between 0.80 and 0.93%) than the soymilk samples (between 0.40 and 0.46%).

Sugar utilization tests indicate that *L. casei* Zhang can metabolize ribose, glucose, mannose, fructose, galactose, sucrose, maltose, and sorbitol, but it cannot or has limited ability to use arabinose, xylose, melibiose, raffinose, and lactose (Menghe et al., 2004). Although bovine milk contains high lactose content and soymilk contains raffinose and stachyose, glucose should be the main carbon source for *L. casei* Zhang in the 2 fermented milk products because of the lack of ability of *L. casei* Zhang to metabolize lactose, raffinose, and stachyose (Menghe et al., 2004). In the present study, the same amounts of glucose were added to the soymilk and bovine milk samples before fermentation. However, besides glucose, *L. casei* Zhang might utilize the sucrose present in soymilk as well, which could be one of the reasons for the faster decrease in pH and faster growth of *L. casei* Zhang in soymilk.

Sequencing the genomes of probiotics such as *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus johnsonii*, and *Lactobacillus plantarum* reveals that they are remarkably deficient in biosynthetic capacities, which are compensated for by their abundant proteolytic systems and extensive capacity for uptake of macromolecules (Pfeiler and Klaenhammer, 2007). In our previous study, we have shown that *L. casei* Zhang

Table 3. Changes in pH and titratable acidity (TA) values in the fermented soymilk and bovine milk samples inoculated with *Lactobacillus casei* Zhang during storage at 4°C for 28 d¹

Inoculation level, cfu/g	0 d			7 d			14 d			21 d			28 d		
	pH	TA (%)		pH	TA (%)		pH	TA (%)		pH	TA (%)		pH	TA (%)	
2 × 10 ⁶															
Soy	4.38 ± 0.02 ^{b,v}	0.55 ± 0.00 ^{b,y}		4.04 ± 0.01 ^{b,w}	0.67 ± 0.00 ^{b,x}		3.91 ± 0.00 ^{b,x}	0.69 ± 0.00 ^{b,x}		3.91 ± 0.00 ^{b,x}	0.88 ± 0.01 ^{b,w}		3.95 ± 0.05 ^{b,x}	0.98 ± 0.06 ^{b,y}	
Bovine	4.47 ± 0.01 ^{a,v}	0.85 ± 0.07 ^{a,y}		4.40 ± 0.02 ^{a,w}	0.91 ± 0.00 ^{a,x,y}		4.36 ± 0.01 ^{a,w}	0.96 ± 0.00 ^{a,w,x}		4.30 ± 0.04 ^{a,x}	1.02 ± 0.02 ^{a,y}		4.18 ± 0.02 ^{a,y}	1.06 ± 0.07 ^{a,y}	
5 × 10 ⁶															
Soy	4.13 ± 0.01 ^{b,v}	0.56 ± 0.00 ^{b,y}		3.94 ± 0.01 ^{b,w}	0.65 ± 0.01 ^{b,x}		3.83 ± 0.00 ^{b,y}	0.76 ± 0.01 ^{b,w}		3.79 ± 0.00 ^{b,z}	0.94 ± 0.01 ^{b,y}		3.90 ± 0.03 ^{b,x}	0.92 ± 0.03 ^{b,y}	
Bovine	4.35 ± 0.02 ^{a,v}	0.91 ± 0.04 ^{a,x}		4.30 ± 0.00 ^{a,w}	0.92 ± 0.03 ^{a,w,x}		4.38 ± 0.03 ^{a,v}	1.01 ± 0.03 ^{a,v}		4.35 ± 0.01 ^{a,v}	1.01 ± 0.01 ^{a,w}		4.24 ± 0.02 ^{a,x}	1.00 ± 0.08 ^{a,w}	
1 × 10 ⁷															
Soy	4.36 ± 0.01 ^{b,v}	0.44 ± 0.00 ^{b,y}		4.13 ± 0.01 ^{b,v}	0.53 ± 0.02 ^{b,x}		3.95 ± 0.00 ^{b,w}	0.60 ± 0.00 ^{b,w}		3.87 ± 0.01 ^{b,y}	0.79 ± 0.01 ^{b,y}		3.93 ± 0.01 ^{b,x}	0.89 ± 0.01 ^{b,y}	
Bovine	4.38 ± 0.01 ^{a,v}	0.93 ± 0.01 ^{a,y}		4.26 ± 0.01 ^{a,x}	0.98 ± 0.01 ^{a,y}		4.38 ± 0.01 ^{a,v}	0.94 ± 0.01 ^{a,v}		4.34 ± 0.00 ^{a,w}	1.01 ± 0.00 ^{a,y}		4.25 ± 0.00 ^{a,x}	1.02 ± 0.09 ^{a,y}	
2 × 10 ⁷															
Soy	4.25 ± 0.01 ^{b,v}	0.54 ± 0.02 ^{b,y}		4.10 ± 0.01 ^{b,w}	0.57 ± 0.00 ^{b,x}		3.94 ± 0.01 ^{b,x}	0.59 ± 0.01 ^{b,x}		3.86 ± 0.02 ^{b,y}	0.82 ± 0.02 ^{b,w}		3.96 ± 0.00 ^{b,x}	0.86 ± 0.01 ^{b,y}	
Bovine	4.43 ± 0.04 ^{a,v}	0.96 ± 0.02 ^{a,x}		4.27 ± 0.01 ^{a,x}	1.01 ± 0.01 ^{a,w}		4.32 ± 0.01 ^{a,w}	1.00 ± 0.02 ^{a,w}		4.25 ± 0.00 ^{a,x}	1.06 ± 0.01 ^{a,y}		4.19 ± 0.01 ^{a,y}	1.08 ± 0.01 ^{a,y}	

^{a,b}Means within a column and inoculation level with different superscript letters are significantly ($P < 0.05$) different.

^{v-z}Means in a row with different superscript letters are significantly ($P < 0.05$) different.

¹Data are means of triplicate determinations ± standard deviation.

Table 4. Changes in the viable counts of *Lactobacillus casei* Zhang in the fermented soymilk and bovine milk samples during storage at 4°C for 28 d¹

Inoculation level, cfu/g	Viable counts				
	0 d	7 d	14 d	21 d	28 d
2 × 10 ⁶					
Soy	9.24 ± 0.02 ^{a,w}	9.42 ± 0.03 ^{a,v}	9.22 ± 0.05 ^{a,w}	9.20 ± 0.03 ^{a,w}	9.25 ± 0.01 ^{a,w}
Bovine	8.59 ± 0.08 ^{b,v}	8.58 ± 0.02 ^{b,v}	8.64 ± 0.16 ^{b,v}	8.23 ± 0.14 ^{b,w}	8.13 ± 0.16 ^{b,w}
5 × 10 ⁶					
Soy	8.86 ± 0.06 ^{a,v}	9.04 ± 0.03 ^{a,v}	8.93 ± 0.06 ^{a,v}	8.96 ± 0.01 ^{a,w}	8.92 ± 0.07 ^{a,w}
Bovine	8.25 ± 0.36 ^{a,v}	8.42 ± 0.27 ^{b,v}	8.10 ± 0.03 ^{b,v}	8.07 ± 0.11 ^{b,v}	8.05 ± 0.02 ^{b,v}
1 × 10 ⁷					
Soy	9.09 ± 0.05 ^{a,w}	9.18 ± 0.04 ^{a,v}	8.94 ± 0.03 ^{a,x}	9.03 ± 0.02 ^{a,w}	9.06 ± 0.03 ^{a,w}
Bovine	8.06 ± 0.58 ^{b,v}	8.41 ± 0.61 ^{b,v}	8.24 ± 0.10 ^{b,v}	8.24 ± 0.03 ^{b,v}	8.10 ± 0.03 ^{b,v}
2 × 10 ⁷					
Soy	9.00 ± 0.06 ^{a,w}	9.17 ± 0.05 ^{a,v}	8.99 ± 0.04 ^{a,w}	8.96 ± 0.07 ^{a,w}	8.94 ± 0.06 ^{a,w}
Bovine	8.47 ± 0.44 ^{a,vw}	8.80 ± 0.13 ^{b,v}	8.68 ± 0.06 ^{b,vw}	8.79 ± 0.54 ^{ab,vw}	8.30 ± 0.06 ^{b,w}

^{a,b}Means within a column and inoculation level with different superscript letters are significantly ($P < 0.05$) different.

^{v-x}Means in a row with different superscript letters are significantly ($P < 0.05$) different.

¹Data are means of triplicate determinations ± standard deviation.

has similar proteolytic activity to *L. acidophilus* NCFM (unpublished data), which was confirmed by the fact that *L. casei* Zhang grew well in different media. The variation in the rate of the acidification might indicate the difference in the proteolytic activity of *L. casei* Zhang in the 2 types of fermented milk products.

The pH buffering capacity of milk is an important determinant of the properties of fermented products, and the contribution of different constituents of milk to the pH buffering properties of various dairy products has been a subject of study for several decades (Whittier, 1929; Upreti et al., 2006; Ahmad et al., 2008). In general, besides weak acids, bases, and their complexes with metal cations, proteins and free amino acids are the major contributors to pH buffering in milk (Upreti et al., 2006). The low final TA values of soymilk indicated that soymilk had lower pH buffering capacity than bovine milk, which might be attributed to the variation in protein composition and physicochemical properties of soy proteins and milk proteins. Glycinin and β -conglycinin are the main proteins present in soybean, constituting about 40 g/100 g and 30 g/100 g of the total soy protein, respectively, whereas bovine milk mainly contains casein and whey proteins. Previous research (Farnworth et al., 2007) reported that soymilk had lower buffering capacity than bovine milk, which was consistent with our results.

Microbiological Analysis. The changes in the viable counts of the fermented soymilk and bovine milk samples during fermentation are shown in Table 2. The fermented soymilk samples had higher viable counts than the fermented bovine milk samples at the end of fermentation. After 6 h of fermentation, *L. casei* Zhang displayed similar growth rates in soymilk and bovine

milk regardless of inoculation rates. However, sharp increases in the viable counts of *L. casei* Zhang were observed in soymilk samples within the next 10 h of fermentation, unlike the slow growth of *L. casei* Zhang in bovine milk. Both the higher viable counts of *L. casei* Zhang and the higher acidification rate in fermented soymilk samples indicated that *L. casei* Zhang might have higher proteolytic activity in soymilk.

Fermentation Characteristics and Viability of *L. casei* Zhang During Storage

pH and TA. The changes in pH and TA of *L. casei* Zhang in the fermented soymilk and bovine milk samples during storage are shown in Table 3. During storage, gradual decreases in pH and increases in TA were observed in both the fermented soymilk and bovine milk samples regardless of the inoculation rates of *L. casei* Zhang. The fermented bovine milk samples had higher TA before storage, ranging from 0.85 to 0.96%, and only slight increases in TA were observed during the 28 d of storage; the final TA values of the samples were in the range of 1.00 to 1.08%. Dramatic increases in TA values in the fermented soymilk samples during storage were observed. The TA values of the fermented soymilk samples before storage were in the range of 0.44 to 0.56%, but the final TA values of the fermented soymilk samples after the 28 d of storage ranged from 0.86 to 0.98%. The continuous decrease in pH in the fermented soymilk and bovine milk during storage showed the occurrence of postacidification, which indicated that *L. casei* Zhang had acid-producing ability at refrigeration temperature. The final pH of the fermented soymilk

Figure 1. Survival of *Lactobacillus casei* Zhang in simulated gastric juice at a) pH 2.0 and b) pH 2.5 at 37°C for 3 h in fermented soymilk and bovine milk samples during storage at 4°C for 28 d. * = survival of pure *L. casei* Zhang in the simulated gastric juice.

after refrigerated storage for 28 d was lower than that of the fermented bovine milk.

The sharp increases in TA values of the fermented soymilk samples indicated that there was dramatic change in buffering capacity during storage. As we pointed out earlier, the pH buffering capacity of milk comes mainly from proteins, free amino acids, weak acids, bases, and their complexes with metal cations (Upreti et al., 2006). Soymilk and bovine milk are unique systems that vary in many aspects such as protein and carbohydrate composition, which results in variation in buffering capacity and fermentation characteristics as observed in the present study. However, more research is needed to elucidate the mechanism of the change in pH and TA of *L. casei* Zhang in the fermented milk samples during fermentation and storage and understand the difference between soy- and milk-based systems.

Microbiological Analysis. The changes in viable counts in the fermented soymilk and bovine milk samples during storage are shown in Table 4. Generally, there were no significant changes in the viable counts of

L. casei Zhang in the 2 types of fermented milk samples at the 4 inoculation rates. For probiotics to perform in vivo, it is essential that the carrier food contains at least 10^6 viable cells of the probiotic microorganism per gram (Vinderola et al., 2000). During the fermentation of soymilk and bovine milk, viable counts of *L. casei* Zhang increased above 10^8 cfu/g and maintained viability during storage at 4°C regardless of inoculation rate, which indicated the probiotic potential of *L. casei* Zhang in various fermented milk products.

Tolerance of *L. casei* Zhang in Various Samples to Simulated GI Tract During Storage

Effect of Storage of Fermented Milk Samples on Viability of *L. casei* Zhang in Simulated Gastric Juice. The effects of storage of the fermented soymilk and bovine milk on the survival rates of *L. casei* Zhang (inoculated at 2×10^7 cfu/g) in simulated gastric juice were studied, and the results are shown in Figure 1. Food is the common delivery system for

Figure 2. Survival of *Lactobacillus casei* Zhang in simulated intestinal juice incubated at 37°C for 4 h in the fermented soymilk and bovine milk samples during storage at 4°C for 28 d. a) After 3 h of incubation in the simulated gastric juice (pH 2.0), *L. casei* Zhang was incubated in simulated intestinal juice for 4 h; b) after 3 h of incubation in simulated gastric juice (pH 2.5), *L. casei* Zhang was incubated in simulated intestinal juice for 4 h. * = survival of pure *L. casei* Zhang in simulated intestinal juice.

probiotic bacteria, and can protect probiotic bacteria from acidic conditions and enhance gastric survival due to the pH increase of the gastric contents resulting from the addition of the food (Huang and Adams, 2004). The survival rates of the control (freshly prepared culture of *L. casei* Zhang) in simulated gastric juice at pH 2.0 and 2.5 were 31 and 69%, respectively, and the delivery of *L. casei* Zhang through the fermented soymilk milk and bovine milk significantly improved the viability of *L. casei* Zhang in the simulated gastric transit to different extents (Figure 1).

Lactobacillus casei Zhang in fermented soymilk and bovine milk samples showed better tolerance to the simulated gastric juice at pH 2.5 (>90%) than at pH 2.0 before and during storage. No significant changes in the viability of *L. casei* Zhang in the simulated gastric juice at pH 2.5 were observed in both the fermented milk samples during storage, and *L. casei* Zhang in the fermented bovine milk samples showed higher survival rate in simulated gastric juice at pH 2.0 than in the fermented soymilk samples. Gradual decreases in the

survival rate of *L. casei* Zhang in the fermented bovine milk in simulated gastric juice of pH 2.0 were reported (from 95 to 71%) during refrigerated storage for 28 d, whereas in the fermented soymilk samples, *L. casei* Zhang showed a low survival rate between 50 and 60% during storage.

Effect of Storage in Various Samples on Viability of *L. casei* Zhang in Simulated Intestinal Juice. Small intestine transit tolerance is essential for probiotic strains to colonize the small intestine. The results of the viability of *L. casei* Zhang after 4 and 8 h of incubation in simulated transit juices are shown in Figures 2 and 3, respectively. The incubation of the samples in simulated gastric juices at pH 2.0 adversely affected the viability of *L. casei* Zhang during the 4- and 8-h incubation in the intestinal juices, and survival rates of *L. casei* Zhang in the soymilk and bovine milk samples after 28 d of storage in the intestinal juices were around 32 and 63%, respectively. In contrast, samples incubated in the simulated transit juices at pH 2.5 showed better viability in both the fermented milk

Figure 3. Survival of *Lactobacillus casei* Zhang in simulated intestinal juice incubated at 37°C for 8 h in the fermented soymilk and bovine milk samples during storage at 4°C for 28 d. a) After 3 h of incubation in simulated gastric juice (pH 2.0), *L. casei* Zhang was incubated in simulated intestinal juice for 8 h; b) after 3 h of incubation in simulated gastric juice (pH 2.5), *L. casei* Zhang was incubated in simulated intestinal juice for 8 h. * = survival of pure *L. casei* Zhang in simulated intestinal juice.

samples during the 4- and 8-h incubation in the intestinal juices (>91 and 75%, respectively), and higher survival rates of *L. casei* Zhang were observed in the bovine milk samples.

The sequenced genomes of intestinal lactobacilli have illustrated how these species adapted to the environment of the GI tract, including stress tolerance, uptake of nutrients, and adhesion to intestinal cells and mucus (Pfeiler and Klaenhammer, 2007). A 2-component regulatory system was identified, which functions in both acid tolerance and proteolysis, indicating an evolutionary link between growth of *L. acidophilus* in milk and acidification (Azcarate-Peril et al., 2005). Through our research, we showed that *L. casei* Zhang had good viability and transit tolerance in fermented milk samples during storage, but more research is needed before industrial production and application of the strain. We have been conducting research on the genomics and proteomics of *L. casei* Zhang, which would bring new insights in the fermentation characteristics and probiotic properties of *L. casei* Zhang.

CONCLUSIONS

The present study showed that *L. casei* Zhang could grow in various foods such as soymilk and bovine milk, and it maintained high viability during 28 d of storage at 4°C. During storage, *L. casei* Zhang showed high tolerance to simulated gastric juice and intestinal juice. Therefore, *L. casei* Zhang showed good probiotic properties and great potential for application in the development of probiotic food products. Variation in fermentation characteristics and transit tolerance of *L. casei* Zhang in fermented soymilk and bovine milk was observed, and further research is needed to understand the difference between soy- and milk-based systems.

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