J. Dairy Sci. 94:3725–3738
doi:10.3168/jds.2010-3737

Some low homogenization pressures improve certain probiotic characteristics of yogurt culture bacteria and *Lactobacillus acidophilus* LA-K[1]

T. Muramalla* and K. J. Aryana*†[2]
*School of Animal Sciences, and
†Department of Food Science, Louisiana State University Agricultural Center, Baton Rouge 70803

ABSTRACT

Lactobacillus delbrueckii ssp. *bulgaricus*, *Streptococcus salivarius* ssp. *thermophilus*, and *Lactobacillus acidophilus* are dairy cultures widely used in the manufacture of cultured dairy products. Commonly used homogenization pressures in the dairy industry are 13.80 MPa or less. It is not known whether low homogenization pressures can stimulate bacteria to improve their probiotic characteristics. Objectives were to determine the effect of homogenization at 0, 3.45, 6.90, 10.34, and 13.80 MPa on acid tolerance, bile tolerance, protease activity, and growth of *L. delbrueckii* ssp. *bulgaricus* LB-12, *S. salivarius* ssp. *thermophilus* ST-M5, and *L. acidophilus* LA-K. The cultures were individually inoculated in cool autoclaved skim milk (4°C) and homogenized for 5 continuous passes. Growth and bile tolerance of samples were determined hourly for 10 h of incubation. Acid tolerance was determined every 20 min for 120 min of incubation. Protease activity was determined at 0, 12, and 24 h of incubation. All homogenization pressures studied improved acid tolerance of *L. delbrueckii* ssp. *bulgaricus* LB-12 but had no beneficial effect on protease activity and had negative effects on growth and bile tolerance. A pressure of 6.90 MPa improved acid tolerance, bile tolerance, and protease activity of *S. salivarius* ssp. *thermophilus* ST-M5, but none of the homogenization pressures studied had an effect on its growth. Homogenization pressures of 13.80 and 6.90 MPa improved acid tolerance and bile tolerance, respectively, of *L. acidophilus* LA-K but had no effect on protease activity and its growth. Some low homogenization pressures positively influenced some characteristics of yogurt culture bacteria and *L. acidophilus* LA-K. Culture pretreatment with some low homogenization pressures can be recommended for improvement of certain probiotic characteristics.

Key words: low homogenization pressure, acid and bile tolerance, protease activity and growth, dairy culture

INTRODUCTION

Lactobacillus bulgaricus and *Streptococcus thermophilus* are 2 microorganisms required in yogurt manufacture according to the definition of yogurt (FDA-DHHS, 2009). Several studies reported yogurt cultures and *Lactobacillus acidophilus* to be probiotics (Guarner et al., 2005; Scholz-Ahrens et al., 2007; Mohammadi and Mortazavian, 2011). *Lactobacillus acidophilus* is widely used as an adjunct culture in approximately 80% of the yogurts being manufactured in the United States (Hutkins, 2006). Heller (2006) reported a 3% increase in sales of cultured dairy products in the United States from 2004 to 2005, generating $9.7 billion in 2005, of which 50% ($4.9 billion) was contributed by sale of yogurt. Dairy Facts (2009) reported a 3.44% increase in sales of yogurt from 2007 to 2008. Granato et al. (2010) and Chandan (1999) explained that the consumption of yogurt was enhanced mainly because of its nutritional value and the beneficial health effects of yogurt cultures.

One of the basic requirements for a culture to be called probiotic is the ability to survive acid and bile conditions in the gastrointestinal tract (Dunne et al., 2001). Acid tolerance of a microorganism has been described as the ability to survive during the passage (transit time of 30–120 min, depending on the type of strain) through the low pH (1.5–3) of the stomach (Lankaputhra and Shah, 1995). Bile tolerance of a microorganism has been described as the ability to survive during the transit time (<3 h, depending on the type of strain) through the high concentration of bile acids (<1.5% wt/vol) in the small intestine and its subsequent colonization in the colon (Lankaputhra and Shah, 1995). Maintaining the functionality of bacterial cultures under gastrointestinal tract conditions (acid and bile) has been one of the major challenges in developing probiotic products (Sandholm et al., 2002).

Protease activity and growth of cultures are important characteristics that affect the texture, taste, and

Received August 17, 2010.
Accepted April 13, 2011.
[1]Approved for publication by the Director of the Louisiana Agricultural Experiment Station as manuscript number 2011-230-5870.
[2]Corresponding author: karyana@agcenter.lsu.edu

shelf life of cultured dairy products (Soda, 1991). The enzymes present in the intracellular components of bacteria are crucial in the proteolysis reaction in fermented products (Gatti et al., 2004). Breakdown or disruption of the cell wall of the bacteria is an essential step to release protease enzymes (Wilkinson and Kilcawley, 2005). Growth and protease activity of dairy cultures are affected during processing and storage of foods due to high temperatures, high pH, and high osmotic pressures (Gardiner et al., 2000; Prasad et al., 2003; Talwalkar and Kailasapathy, 2003).

Homogenization is a fluid mechanical process that involves the breakup of particles or droplets into micron sizes and the creation of a stable dispersion or emulsion (Diels and Michiels, 2006). Three main mechanisms by which homogenization pressure exerts its action on fluid have been described; namely, turbulence (Doulah and Hammond, 1975), cavitation (Deshimaru, 1994), and impingement (Kleinig and Middelberg, 1996). Homogenization causes various changes in the cell wall of microorganisms, leading to changes such as proteolysis, lipolysis, and glycolysis (Gatti et al., 2004; Vannini et al., 2004; Lanciotti et al., 2007).

Several factors influence microbial growth with homogenization: temperature (Floury et al., 2000), type of bacteria (gram-positive or gram-negative; Vachon et al., 2002), number of homogenization passes (Bevilacqua et al., 2009), composition of the medium (Kheadr et al., 2002), and homogenization pressure. Homogenization pressures of 13.80 MPa or less are commonly used in dairy processing (Pandolfe, 1982).

Homogenization pressure is defined as the overall effect of compression in the intensifier (homogenizer) and the flow through the fittings and piping of the system that would lead to an effective stress on a microbial population (Donsì et al., 2009). Bevilacqua et al. (2009) reported an increase in the log reduction of lactic acid bacteria (*Lactobacillus plantarum*, *Lactobacillus brevis*, and *Bifidobacterium coagulans*) from 0.7 to 2.4 log cfu/mL by increasing the pressure from 50 to 150 MPa. Recently, Donsì et al. (2009) reported that inactivation of *Escherichia coli* increased from 1 to 5 log cycles upon increasing the homogenization pressure from 100 to 300 MPa. Coskun (2006) reported that mesophilic lactic acid bacteria (*Lactococcus lactis* ssp. *lactis* and *Lactococcus lactis* ssp. *cremoris*) homogenized at 30 MPa exhibited higher proteolysis than thermophilic lactic acid bacteria (*L. delbrueckii* ssp. *bulgaricus* and *S. thermophilus*) homogenized at the same pressure.

Depending on the range of pressures applied, homogenization can be broadly classified into high and low homogenization pressures. Pressures >50 MPa are generally considered as high pressure (Vachon et al., 2002; Vannini et al., 2004; Diels and Michiels, 2006). Several previous studies (Lanciotti et al., 1996; Vannini et al., 2004; Brinez et al., 2006; Diels and Michiels, 2006) have shown the effect of high homogenization pressures on the growth of pathogens and spoilage microorganisms, but information about low homogenization pressures (≤13.80 MPa) on bacterial probiotic characteristics is lacking. The hypothesis was that commonly used low homogenization pressures could stimulate bacteria to improve their probiotic characteristics. The objectives were to determine the effect of 0, 3.45, 6.90, 10.34, and 13.80 MPa on acid tolerance, bile tolerance, protease activity, and growth of *L. delbrueckii* ssp. *bulgaricus* LB-12, *S. salivarius* ssp. *thermophilus* ST-M5, and *L. acidophilus* LA-K.

MATERIALS AND METHODS

Experimental Design

Skim milk was sterilized by autoclaving at 121°C for 15 min. This sterile milk was cooled to 4°C and individually inoculated with 0.1% (vol/vol) *L. delbrueckii* ssp. *bulgaricus* LB-12 (hereafter **LB-12**), *S. salivarius* ssp. *thermophilus* ST-M5 (hereafter **ST-M5**), or *L. acidophilus* LA-K (hereafter **LA-K**; Chr. Hansen Laboratory, Milwaukee, WI). The treatments were homogenization pressures of 3.45, 6.90, 10.34, and 13.80 MPa. The control was the sample passed through homogenizer at 0 MPa. The homogenization pressures were randomized for all 3 replications.

The control and the treated (homogenized) samples were tested for acid tolerance, bile tolerance, protease activity, and growth. Growth was determined by plating the homogenized cultures every hour for 10 h. Bile tolerance of the cultures was determined by growing the homogenized cultures in presence of bile and plating every hour for 10 h. Acid tolerance was determined by inoculating the homogenized cultures into acidified broth and plating every 20 min up to 120 min. Protease activity of the homogenized cultures was determined by measuring optical density (absorbance value) at 0, 12, and 24 h of incubation of the samples. Three replications were conducted with replications as blocks. The experiment was conducted as a completely randomized block design with repeated measurements.

Homogenizer

A Gaulin homogenizer (Manton-Gaulin Manufacturing Company Inc., Everett, MA) was used for the experiment. The Gaulin homogenizer is a 2-stage homogenizer. The first stage breaks up the fat globules into smaller particles and the second stage prevents the coalescence of the broken fat globules (Middelberg,

1995). In the present experiment, only the first stage was used for all the pressures applied on the samples to maintain a simple experimental design and to avoid the combined effect of 2-stage homogenization.

A soap solution was run through the homogenizer for 30 min. Four hundred milliliters of active chlorine (3,333 ppm) in 10 L of water was passed through the homogenizer for 2 min. Hot autoclaved water was passed through the sanitized homogenizer, followed by passage of cooled autoclaved water, which was collected and serially diluted and plated using Lactobacilli MRS agar (Fisher Scientific, Fair Lawn, NJ) and Aerobic Count Plate Petrifilm (3M Microbiology Products, St. Paul, MN) individually. The MRS Petri plates were incubated anaerobically for 72 h at 43°C (Simova et al., 2006) and Aerobic Count Plate Petrifilms were incubated aerobically at 32°C for 48 h and colonies were counted (Champagne et al., 2009). Counts were zero, indicating that the sanitization method successfully sterilized the homogenizer.

Preparation of Media

S. thermophilus Agar. The *S. thermophilus* (**ST**) agar was prepared according to the method described by Dave and Shah (1996). Ten grams of Bacto tryptone (Difco, Becton-Dickinson and Co., Sparks, MD), 10 g of sucrose (Amresco, Solon, OH), 5 g of Bacto yeast extract (Difco), and 2 g of dipotassium phosphate (K_2HPO_4; Fisher Scientific) were dissolved in 1 L of distilled water. The pH of the resulting mixture was adjusted to 6.8 ± 0.1 using 1 *M* HCl. Six milliliters of 0.5% bromocresol purple (Fisher Scientific) and 12 g of agar (Fisher Scientific) were added to the medium. The medium was boiled and sterilized at 121°C for 15 min.

Lactobacilli MRS Agar and pH-Modified MRS Agar (pH 5.2). Fifty-five grams of de Man, Rogosa, and Sharpe (**MRS**) broth (Difco) and 15 g of agar (Fisher Scientific) were dissolved in 1 L of distilled water, boiled, and sterilized at 121°C for 15 min (Dave and Shah, 1996). The pH of MRS agar (Difco) was adjusted to 5.2 using 1 *M* HCl (Dave and Shah, 1996).

Analytical Procedures

Acid Tolerance. The acid tolerance of the 3 cultures was determined by using the method proposed by Pereira and Gibson (2002) with slight modifications. The control and homogenized samples were inoculated into acidified MRS broth (Difco) previously adjusted to pH 2 using 1 *M* HCl. The inoculated acidified MRS broth was incubated at 37°C for LA-K, 43°C for LB-12, and 37°C for ST-M5 for 120 min. One milliliter of the inoculated broth was serially diluted in peptone water (0.1% wt/vol) and pour-plated every 20 min for 120 min. The cultures LA-K, LB-12, and ST-M5 were enumerated using Lactobacilli MRS agar, pH-modified Lactobacilli MRS agar, and ST agar, respectively (Dave and Shah 1996). The Petri plates were incubated anaerobically at 37°C for 72 h for LA-K, anaerobically at 43°C for 72 h for LB-12, and aerobically at 37°C for 24 h for ST-M5. After the incubation period, the colonies were counted.

Bile Tolerance. Bile tolerance was determined according to method proposed by Pereira and Gibson (2002) with slight modifications. The bile tolerance of the 3 cultures was analyzed in MRS-Thio broth: MRS broth supplemented with 0.3% (wt/vol) Oxgall (bovine bile; US Biological, Swampscott, MA) and 0.2% (wt/vol) sodium thioglycolate (Acros Organics, Fair Lawn, NJ). Oxgall was added to test bile tolerance of the bacteria and sodium thioglycolate was used in the broth as an oxygen scavenger. The MRS-Thio broth was inoculated at 10% (vol/vol) with control and homogenized cultures separately and incubated at 37°C for LA-K, 43°C for LB-12, and 37°C for ST-M5 for 10 h. Each hour for 10 h, 1 mL of the inoculated broth was serially diluted into peptone water (0.1% wt/vol) and pour-plated. The cultures LA-K, LB-12, and ST-M5 were enumerated using Lactobacilli MRS agar, pH-modified Lactobacilli MRS agar, and ST agar, respectively (Dave and Shah, 1996). The plates were incubated anaerobically at 37°C for 72 h for LA-K, anaerobically at 43°C for 72 h for LB-12, and aerobically at 37°C for 24 h for ST-M5. After the incubation period, the colonies were counted.

Protease Activity. The protease activity of 3 cultures was determined by *o*-phthaldialdehyde (**OPA**) spectrophotometric method proposed by Oberg et al. (1991) with slight modification. The modifications were use of fluid skim milk instead of reconstituted NDM and running the sample immediately after preparation without prior freezing at −70°C. The control and homogenized samples were incubated at 40°C for 0, 12, and 24 h. After incubation, 2.5 mL from each sample was mixed with 1 mL of distilled water individually and was transferred into each of the test tubes containing 5 mL of 0.75 *N* TCA (Fisher Scientific) and the test tubes were vortexed. After sitting at room temperature for 10 min, the acidified samples were filtered through a Whatman no. 2 filter paper (Clifton, NJ). Duplicate aliquots from each TCA filtrate were analyzed by the OPA procedure using a spectrophotometer (Nicolet Evolution 100, Thermo Scientific, Madison, WI). The OPA final solution was prepared by combining 25 mL of 100 m*M* sodium borate (Fisher Scientific), 2.5 mL of 20% (wt/wt) SDS (Fisher Scientific), 40 mg of OPA reagent (Alfa Aesar, Ward Hill, MA) dissolved in 1

mL of methanol (Sigma, St. Louis, MO) and 100 μL of β-mercaptoethanol (Sigma), and diluting to a final volume of 50 mL with distilled water. One hundred fifty microliters of each TCA filtrate was mixed with 3 mL of OPA reagent in a 3-mL cuvette, and the absorbance at 340 nm was read. Absorbance of the OPA final solution with the noninoculated sterile skim milk was subtracted from each sample reading. The OPA reagent was used as a blank to calibrate the spectrophotometer.

Growth. Growth of LB-12, ST-M5, and LA-K was determined by the method proposed by Lin and Young (2000) with slight modifications. Control and homogenized samples were inoculated at 10% (vol/vol) separately into MRS broth, which was previously autoclaved at 121°C for 15 min with pH 6.5 ± 0.2. The inoculated broths were incubated at 37°C for LA-K, 43°C for LB-12, and 37°C for ST-M5 for 10 h. Each hour for 10 h, 1 mL of the inoculated broth was serially diluted in peptone water (0.1% wt/vol) and pour-plated. The cultures LA-K, LB-12, and ST-M5 were enumerated using Lactobacilli MRS agar, pH-modified Lactobacilli MRS agar, and ST agar, respectively (Dave and Shah, 1996). The plates were incubated anaerobically at 37°C for 72 h for LA-K, anaerobically at 43°C for 72 h for LB-12, and aerobically at 37°C for 24 h for ST-M5. After the incubation period, the colonies were counted.

Statistical Analysis

Data were analyzed using Proc Mixed of SAS (SAS Institute, 2003). The homogenization pressure treatments were compared among each other and with the control (0 MPa). Differences of least squares means were used to determine significant differences at $P < 0.05$ for main effect (homogenization pressure) and interaction effect (homogenization pressure × time). Data are presented as mean ± standard error of the means. Significant differences were determined at $\alpha = 0.05$. Significant differences ($P < 0.05$) among the homogenization pressures (3.45, 6.90, 10.34, and 13.80 MPa) and the control (0 MPa) were analyzed using Tukey's adjustment.

RESULTS AND DISCUSSION

Acid Tolerance

L. delbrueckii ssp. bulgaricus LB-12. The viability of LB-12 subjected to different low homogenization pressures when incubated in acid conditions is presented in Figure 1A. A significant ($P < 0.05$) interaction was observed between the homogenization pressures and time (min; Table 1). In the control group, a significant ($P < 0.05$) decrease occurred in viable counts every 20 min, and the viable count was zero after 60 min of incubation (Figure 1A). In general, the viable counts of LB-12 subjected to various pressures were significantly ($P < 0.05$) higher in every 20-min interval of incubation compared with the control, and this trend was observed throughout 120 min of incubation (Table 2). More importantly, LB-12 subjected to pressures of 13.80 and 10.34 MPa exhibited almost no decline in viable counts throughout 120 min of incubation period (Figure 1A).

Homogenization pressures had a significant ($P < 0.05$) effect on acid tolerance of LB-12 (Table 1). The acid tolerance of LB-12 subjected to pressures of 3.45, 6.90, 10.34 and 13.80 MPa was significantly ($P < 0.05$) higher than that of the control (0 MPa; Table 3). A significant ($P < 0.05$) difference existed among acid tolerances of LB-12 subjected to various homogenization pressures (Table 3). The acid tolerance of the control (0 MPa) was the lowest ($P < 0.05$), and that subjected to 13.80 MPa was the highest ($P < 0.05$), followed by those exposed to 10.34, 3.45, and 6.90 MPa. The LB-12 homogenized at 10.34 MPa was found to exhibit significantly ($P < 0.05$) higher acid tolerance compared with that at 6.90 MPa.

S. salivarius ssp. thermophilus ST-M5. The acid tolerance of ST-M5 (expressed as log cfu/mL) at different homogenization pressures is shown in Figure 1B. The interaction between homogenization pressures and time (min) was not significant ($P > 0.05$; Table 1), but homogenization pressure had a significant ($P < 0.05$) effect (Table 1). Acid tolerance of ST-M5 subjected to different homogenization pressures was significantly ($P < 0.05$) higher than the control (Table 3). Acid tolerance of ST-M5 subjected to 3.45 MPa was higher ($P < 0.05$) than that subjected to 6.90 MPa (Table 3). The homogenized culture subjected to different pressures exhibited viable counts after 120 min of incubation in acid conditions (Figure 1B). The acid tolerance of ST-M5 subjected to 3.45 MPa was higher ($P < 0.05$) than that at 6.90 MPa but not different from that at 10.34 and 13.80 MPa. The acid tolerance of control (0 MPa) was the lowest ($P < 0.05$).

L. acidophilus LA-K. The acid tolerance of LA-K (expressed as log cfu/mL) at different homogenization pressures is shown in Figure 1C. The interaction between homogenization pressures and time (min) was not significant ($P > 0.05$), but homogenization pressure had a significant effect ($P < 0.05$) on acid tolerance (Table 1). The acid tolerance of LA-K subjected to homogenization pressure of 13.80 MPa was the highest ($P < 0.05$) (Table 3). The acid tolerance of LA-K subjected to homogenization pressure of 10.34 MPa was significantly ($P < 0.05$) higher than that at 6.90, 3.45 or 0 MPa, which were not significantly different from each other. Homogenized cultures of LA-K subjected to

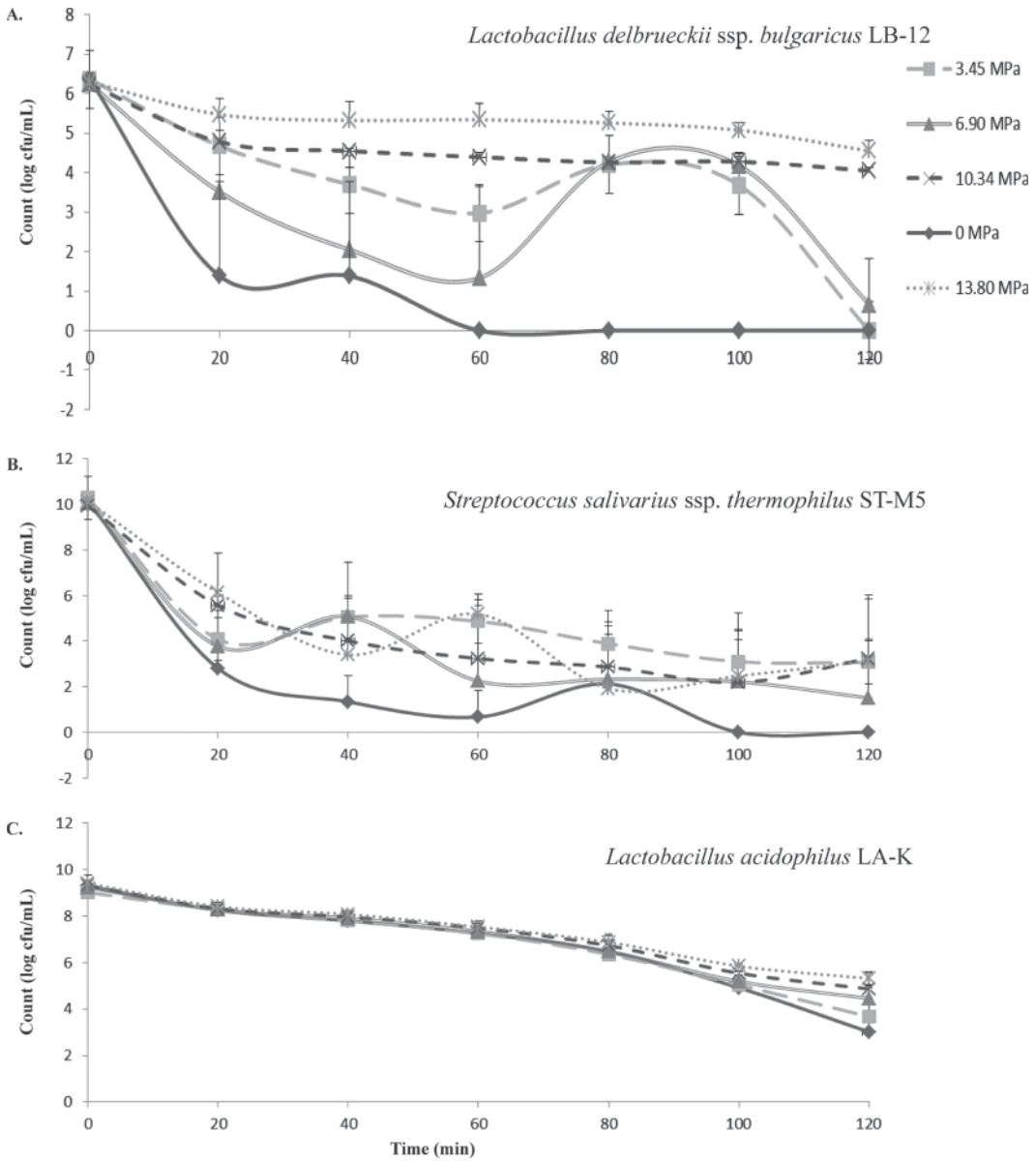

Figure 1. Acid tolerance of homogenized cultures of *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12, *Streptococcus salivarius* ssp. *thermophilus* ST-M5, and *Lactobacillus acidophilus* LA-K at different pressures.

different pressures, as well as the control, were acid tolerant until the end of the 120 min of incubation in acid conditions; however, a significant ($P < 0.05$) decrease in viable counts was observed after each incubation time interval of 20 min.

Acid tolerance is strain-dependent (Tuomola et al., 2001); Liong and Shah (2005) observed that *L. acidophilus* exhibited more acid tolerance than *Lactobacillus casei*. They reported that *L. acidophilus* counts decreased by 1.72 log cycles when incubated at pH 2 for 120 min, whereas, *L. casei* counts decreased by 3.04 log cycles. Mean log reductions of the viable counts of the homogenized cultures obtained by subtracting counts at 0 min from 120 min for pH 2 at different homogenization pressures are reported in Table 4. In Table 4, a higher negative number means high bacterial death and lower negative number means low bacterial death. Comparing the viability of the bacteria from 0 to 13.80 MPa for *L. acidophilus* indicates a decrease in viability (−3.87 to −4.21), for *L. delbrueckii* ssp. *bulgaricus* an increase in viability (−6.52 to −1.60), and for *S. thermophilus* an increase in viability (−10.25 to −4.29). These findings indicate that increase in homogenization pressure from 0 to 13.80 MPa resulted in increased acid tolerance of the yogurt cultures (LB-12 and ST-M5). Coskun (2006) found that homogenization of lactic acid

Table 1. The *P*-value > F-value of homogenization pressure, time, and their interaction for acid tolerance, bile tolerance, protease activity, and growth of *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12, *Streptococcus salivarius* ssp. *thermophilus* ST-M5, and *Lactobacillus acidophilus* LA-K

Effect	*L. delbrueckii* ssp. *bulgaricus* LB-12	*S. thermophilus* ST-M5	*L. acidophilus* LA-K
Acid tolerance			
Pressure	<0.0001	<0.0001	0.0117
Time	<0.0001	<0.0001	<0.0001
Pressure × time	<0.0001	0.4497	0.7587
Bile tolerance			
Pressure	<0.0001	0.0007	0.0085
Time	<0.0001	<0.0001	<0.0001
Pressure × time	0.7941	0.8608	0.2665
Protease activity			
Pressure	0.4339	0.0004	0.333
Time	<0.0001	0.0004	<0.0001
Pressure × time	0.7139	0.3871	0.4436
Growth			
Pressure	<0.0001	0.2186	0.3334
Time	0.0292	<0.0001	<0.0001
Pressure × time	0.9296	0.6221	0.2758

bacterial cultures (*Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris*, *L. delbrueckii* ssp. *bulgaricus*, *S. thermophilus*, and *Lactobacillus helveticus*) to a high pressure of 30 MPa decreased the acid production of the cultures because of cell damage.

Shah and Jelen (1990) reported that at pH 1.5, *L. delbrueckii* ssp. *bulgaricus* and *L. acidophilus* proved to be more acid resistant than *S. thermophilus* strains. Dunne et al. (2001) reported that *L. acidophilus* was more acid resistant at pH values of 1.2 and 2.5 for 30 min compared with *Bifidobacterium* spp., which did not tolerate acid conditions after 5 min. In our study, LA-K and LB-12 were found to be more acid tolerant than ST-M5 at 0 MPa, as indicated by log difference values of −3.87, −6.52, and −10.25 log cfu/mL, respectively (Table 4). Moreover, LB-12 exhibited better acid tolerance than LA-K and ST-M5 after homogenization at 13.80 MPa, as indicated by log difference values of −1.60, −4.21, and −4.29 log cfu/mL, respectively.

Bile Tolerance

L. delbrueckii ssp. bulgaricus LB-12. Bile tolerance of LB-12 (expressed as log cfu/mL) at different homogenization pressures is shown in Figure 2A. The interaction between homogenization pressures and time was not significant ($P > 0.05$) (Table 1), but homogenization pressure had a significant ($P < 0.0001$) effect on bile tolerance of LB-12 (Table 1). The bile tolerance of LB-12 at 0 MPa was significantly ($P < 0.05$) higher than that of cultures subjected to different homogenization pressures (Table 3). The bile tolerance of LB-12 subjected to 3.45 and 6.90 MPa was significantly ($P < 0.05$) higher than that subjected to 13.80 MPa (Table 3). Strain LB-12 was found to be bile tolerant throughout 10 h of incubation in bile conditions (Figure 2A). However, LB-12 subjected to low homogenization pressures, including 0 MPa, exhibited a decline in viable counts at the end of 10 h of incubation (Table 4). The bile tolerance of LB-12 at 0 MPa was the highest ($P < 0.05$) followed by LB-12 at 6.90, 3.45, 10.34, and 13.80 MPa (Table 3). The bile tolerance of LB-12 subjected to 13.80 MPa was significantly ($P < 0.05$) lower than that of 0, 3.45, and 6.90 MPa (Table 3).

S. salivarius ssp. thermophilus ST-M5. The bile tolerance of ST-M5 at different homogenization pressures is shown in Figure 2B. The interaction between homogenization pressure and time was not significant ($P > 0.05$), whereas homogenization pressures had a

Table 2. The *P*-value > F-value of acid tolerance of *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12 at various pressures compared with control (0 MPa)

Pressure (MPa)	Time interval during incubation period of 120 min					
	20 min	40 min	60 min	80 min	100 min	120 min
3.45	<0.0001	0.0059	0.0005	<0.0001	<0.0001	1.0000
6.90	0.0110	0.4162	0.1064	<0.0001	<0.0001	0.4162
10.34	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	<0.0001
13.80	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

Table 3. Least squares means for growth of *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12, *Streptococcus salivarius* ssp. *thermophilus* ST-M5, and *Lactobacillus acidophilus* LA-K as influenced by homogenization pressure

Pressure (MPa)	*L. delbrueckii* ssp. *bulgaricus* LB-12	*S. thermophilus* ST-M5	*L. acidophilus* LA-K
Acid tolerance			
0 (control)	1.304[d]	2.435[c]	6.712[c]
3.45	3.660[c]	4.900[a]	6.771[c]
6.9	3.182[c]	3.877[b]	6.982[c]
10.34	4.650[b]	4.427[ab]	7.173[b]
13.8	5.333[a]	4.618[ab]	7.347[a]
Bile tolerance			
0 (control)	8.957[a]	10.596[c]	8.966[bc]
3.45	8.841[b]	10.728[b]	8.992[abc]
6.9	8.846[b]	10.790[a]	9.019[a]
10.34	8.830[bc]	10.713[b]	8.996[ab]
13.8	8.736[c]	10.813[a]	8.957[c]
Protease activity			
0 (control)	NS	0.025[b]	NS
3.45	NS	0.031[b]	NS
6.9	NS	0.046[a]	NS
10.34	NS	0.045[a]	NS
13.8	NS	0.027[b]	NS
Growth			
0 (control)	9.350[a]	NS	NS
3.45	9.310[ab]	NS	NS
6.9	9.266[b]	NS	NS
10.34	9.300[b]	NS	NS
13.8	9.172[c]	NS	NS

[a–d]Means within a column not containing a common superscript differ ($P < 0.05$).

significant ($P < 0.001$) effect on bile tolerance of ST-M5 (Table 1). The bile tolerance of ST-M5 subjected to 3.45, 6.90, 10.34, and 13.80 MPa was significantly ($P < 0.05$) higher than that of the control (Table 3). In addition, a significant ($P < 0.05$) difference was observed in bile tolerance of ST-M5 subjected to different homogenization pressures (Table 3). The bile tolerance of ST-M5 subjected to 13.80 and 6.90 MPa

Table 4. Mean log difference[1] (log cfu/mL) in the viable counts of *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12, *Streptococcus salivarius* ssp. *thermophilus* ST-M5, and *Lactobacillus acidophilus* LA-K in the homogenized cultures[2]

Pressure (MPa)	*L. delbrueckii* ssp. *bulgaricus* LB-12	*S. thermophilus* ST-M5	*L. acidophilus* LA-K
Acid tolerance			
0 (control)	−6.52	−10.25	−3.87
3.45	−6.62	−4.91	−4.26
6.9	−4.56	−6.41	−4.44
10.34	−2.25	−4.46	−4.52
13.8	−1.6	−4.29	−4.21
Bile tolerance			
0 (control)	−0.439	−0.081	0.747
3.45	−0.83	−0.148	0.876
6.9	−0.813	0.021	0.967
10.34	−0.663	−0.02	0.948
13.8	−0.567	0.013	0.787
Growth			
0 (control)	−0.117	1.764	0.737
3.45	−0.08	1.787	0.68
6.9	−0.011	1.706	0.964
10.34	0.038	1.848	0.827
13.8	−0.099	1.819	0.698

[1]Mean log difference for acid tolerance = (viable log cfu/mL at 2 h) – (viable log cfu/mL at 0 h). Mean log difference for bile tolerance or growth = (viable log cfu/mL at 10 h) – (viable log cfu/mL at 0 h).

[2]Obtained by subtracting initial counts of viable bacteria at the start of the incubation period from those at the end of incubation period for the same microorganism at the same homogenization pressure.

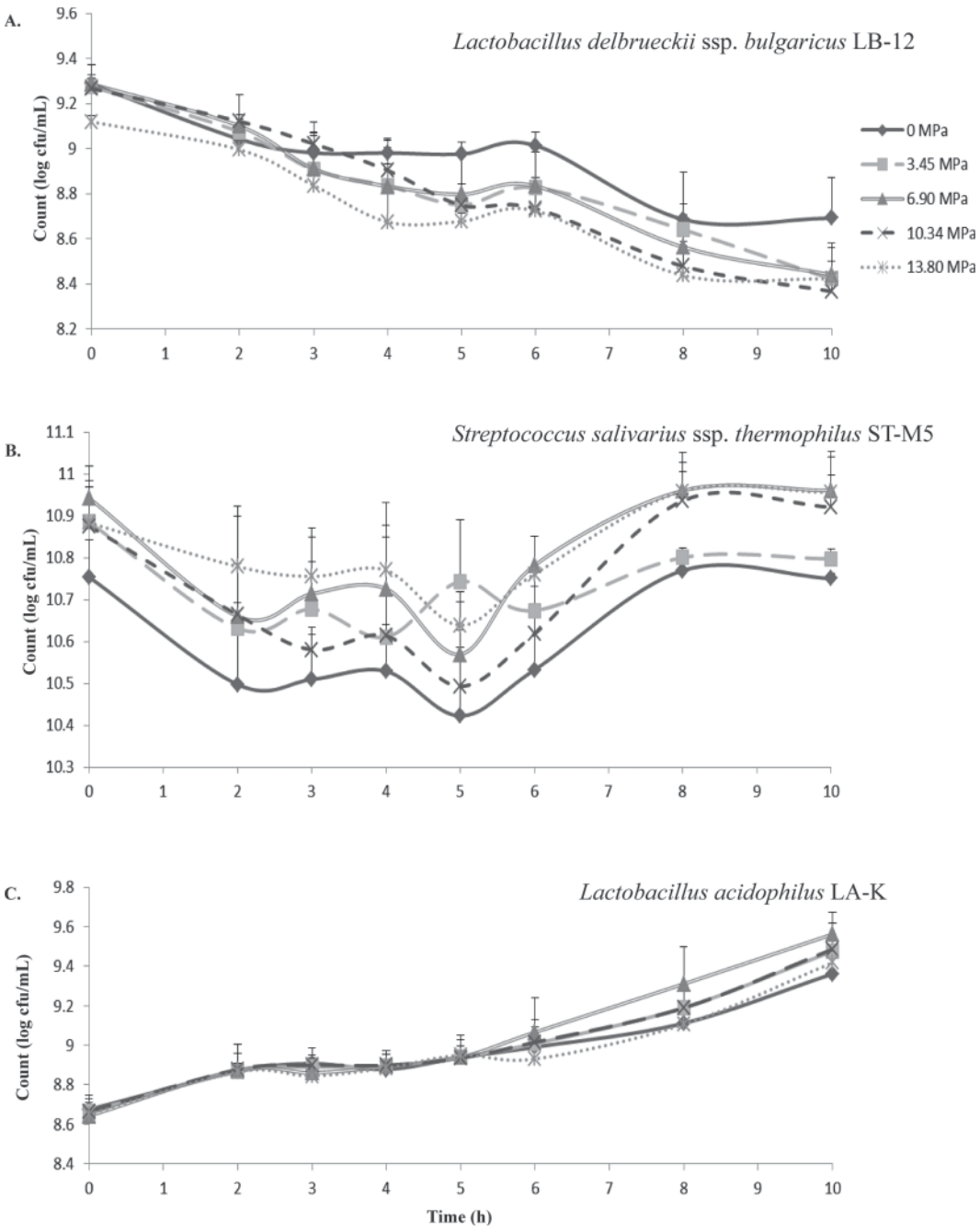

Figure 2. Bile tolerance of homogenized cultures of *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12, *Streptococcus salivarius* ssp. *thermophilus* ST-M5, and *Lactobacillus acidophilus* LA-K at different pressures.

was significantly ($P < 0.05$) higher than that of ST-M5 subjected to 3.45 and 10.34 MPa (Table 3). Strain ST-M5 exhibited no decrease in viable counts between 0 and 10 h of incubation in presence of bile (Figure 2B). Bile tolerance of the control (0 MPa) ST-M5 was the lowest ($P < 0.05$; Table 3).

***L. acidophilus* LA-K.** Bile tolerance of LA-K at different homogenization pressures is shown in Figure 2C. The interaction between homogenization pressures and time was not significant ($P > 0.05$, Table 1), whereas homogenization pressures had a significant ($P < 0.01$) effect on bile tolerance of LA-K (Table 1). The bile tolerance of LA-K subjected to 6.90 MPa was significantly ($P < 0.05$) higher than that of LA-K subjected to 0 and 13.80 MPa (Table 3). Strain LA-K exhibited high tolerance to bile conditions, with an increase in

viable counts of the homogenized culture during 10 h of incubation in bile conditions (Table 4).

In the present study, we found that *L. acidophilus* exhibited similar growth patterns in the presence or absence of bile acids. Similar findings were reported by Liong and Shah (2005) who studied bile tolerance of different strains of *Lactobacillus* species and found that *L. acidophilus* exhibited similar growth patterns in the presence or absence of bile acid (Oxgall). In the present study, *L. acidophilus* showed the highest bile tolerance followed by *S. thermophilus*, and *L. delbrueckii* ssp. *bulgaricus* exhibited least bile tolerance (Table 4). We found that homogenization pressures had significant ($P < 0.05$) effects on bile tolerance of these bacteria (Table 1). Homogenization pressures significantly ($P < 0.05$) improved the bile tolerance of *S. thermophilus* (Table 3) and significantly ($P < 0.05$) decreased the bile tolerance of *L. delbrueckii* ssp. *bulgaricus* (Table 3), indicating that different bacteria have a different bile tolerance responses to the same homogenization pressures. Shah and Jelen (1990) attributed the increased bile tolerance of *L. acidophilus* to its rigid cell wall. Our results indicated that the bile tolerance of *L. acidophilus* increased with increasing homogenization pressure from 0 MPa to 3.45, 6.90, 10.34, and 13.80 MPa (Table 4). These results indicated that, in addition to the rigid cell wall (Shah and Jelen, 1990), other factors could be responsible for increased bile tolerance of *L. acidophilus* when subjected to homogenization pressures.

Protease Activity

L. delbrueckii ssp. bulgaricus LB-12. The protease activity of LB-12 expressed as optical density (absorbance values) at different homogenization pressures is shown in Figure 3A. The interaction between homogenization pressure and time was not significant ($P > 0.05$; Table 1), and homogenization pressure did not have a significant ($P > 0.05$) effect on the protease activity (Table 1).

S. salivarius ssp. thermophilus ST-M5. The protease activity of ST-M5 expressed as optical density (absorbance values) at different homogenization pressures is shown in Figure 3B. The interaction between homogenization pressures and time was not significant ($P > 0.05$; Table 1), but homogenization pressure had a highly significant ($P < 0.001$) effect on protease activity of ST-M5 (Table 1). The protease activity of ST-M5 subjected to pressures of 6.90 and 10.34 MPa was significantly ($P < 0.05$) higher than that of the control and ST-M5 subjected to 3.45 and 13.80 MPa (Table 3). Homogenization pressures of 6.90 and 10.34 MPa significantly ($P < 0.05$) improved the protease activity of ST-M5.

L. acidophilus LA-K. The protease activity of LA-K expressed as optical density (absorbance values) at different homogenization pressures is shown in Figure 3C. The interaction between the homogenization pressures and time was not significant ($P > 0.05$; Table 1), and homogenization pressure had no significant ($P > 0.05$) effect on LA-K (Table 1).

In this study, low homogenization pressures did not result in any significant ($P > 0.05$) change in protease activity of *L. delbrueckii* ssp. *bulgaricus*. Previously, Gatti et al. (2004) reported a decrease in aminopeptidase activity of *L. delbrueckii* ssp. *bulgaricus* after subjecting it to 8.82 MPa using a pressure cell. Use of a pressure cell versus a homogenizer to apply pressure probably explains the difference in findings. Low homogenization pressures did not influence protease activity of *L. acidophilus* and *L. delbrueckii* ssp. *bulgaricus* (Table 1), but influenced the protease activity of *S. thermophilus* (Table 1). Homogenization pressures of 6.90 and 10.34 MPa improved the protease activity of *S. thermophilus* significantly ($P < 0.05$; Table 3), as determined by the Pr > t values of the differences of the least squares means. Coskun (2006) also stated that addition of attenuated (homogenized) cultures subjected to the homogenization pressure of 30 MPa enhanced the proteolytic activity of the lactic acid bacteria (*L. lactis*, *L. cremoris*, *L. delbrueckii* ssp. *bulgaricus*, and *S. thermophilus*). Differences in protease activity of homogenized cultures determined by subtracting the absorbance values at 0 h from 12 h at different homogenization pressures are shown in Table 5. In Table 5, a positive (negative) number indicates an increase (decrease) in protease activity and the higher the number, the higher the protease activity is. In our experiments, *L. delbrueckii* ssp. *bulgaricus* exhibited the highest protease activity after 12 h of incubation compared with *L. acidophilus* and *S. thermophilus*; *S. thermophilus* exhibited the lowest protease activity (Table 5). This is in accordance with the results reported by Shah and Jelen (1990) that *L. delbrueckii* ssp. *bulgaricus* exhibited high β- galactosidase activity compared with *S. thermophilus* and *L. acidophilus*.

Growth

L. delbrueckii ssp. bulgaricus LB-12. Growth of LB-12 (expressed as log cfu/mL) at different homogenization pressures is shown in Figure 4A. The interaction between the homogenization pressures and time was not significant ($P > 0.05$); however, homogenization pressure had a significant ($P < 0.0001$) effect on growth of LB-12 (Table 1). Homogenization pressures of 6.90, 10.34, and 13.80 MPa resulted in significantly ($P < 0.05$) decreased growth compared with the control,

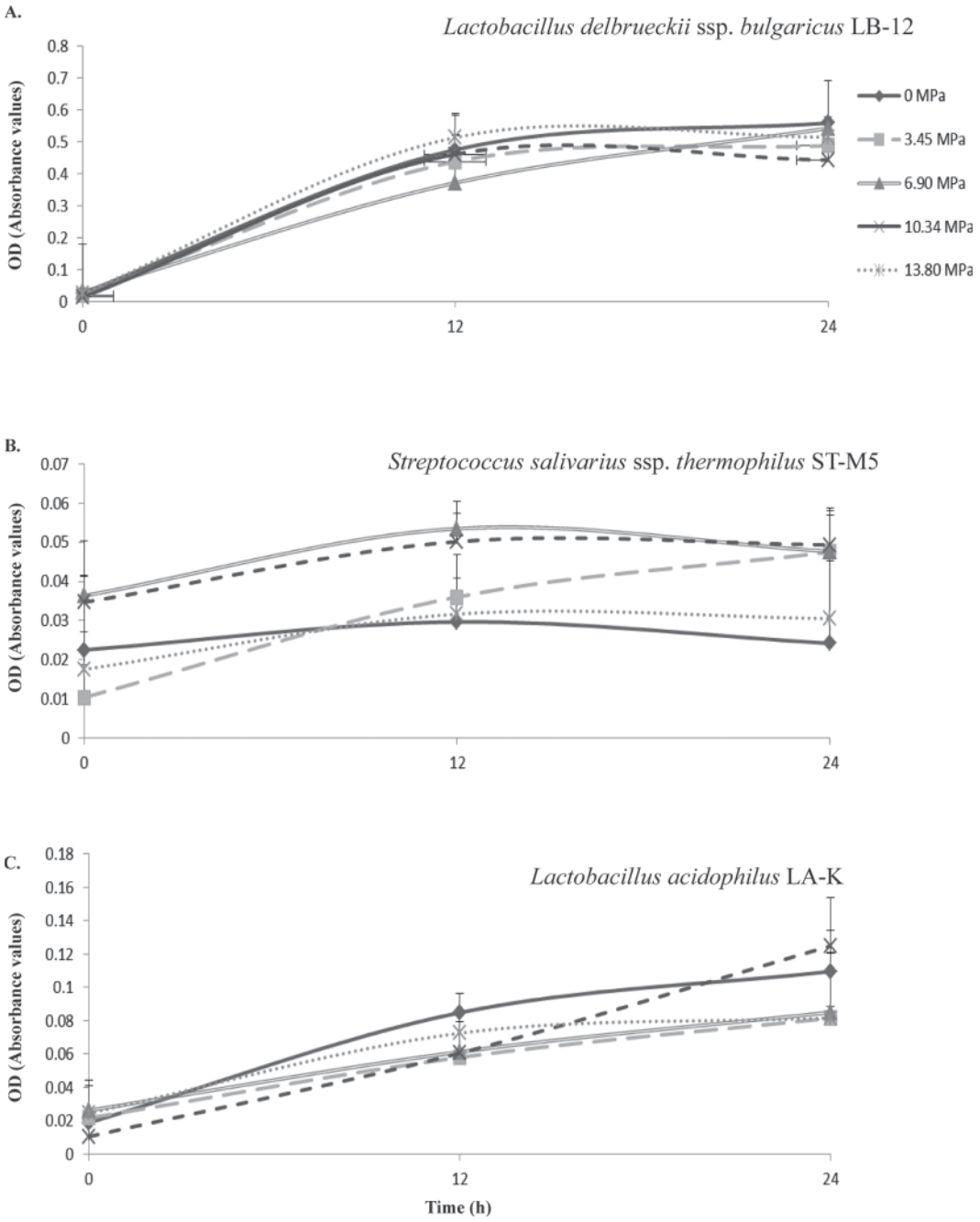

Figure 3. Protease activity of the homogenized cultures of *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12, *Streptococcus salivarius* ssp. *thermophilus* ST-M5, and *Lactobacillus acidophilus* LA-K at different pressures.

whereas the homogenization pressure of 3.45 MPa did not result in significant change in growth compared with control (Table 3). Furthermore, the growth of homogenized cultures subjected to 3.45, 6.90, and 10.34 MPa were significantly ($P < 0.05$) higher than growth of LB-12 subjected to 13.80 MPa (Table 3). The logarithmic phase of the bacterium was not observed during 10 h of incubation in any of the pressures under study, including the control (Figure 4A).

S. salivarius ssp. thermophilus ST-M5. Growth of ST-M5 (expressed as log cfu/mL) at different homogenization pressures is shown in Figure 4B. The interaction between homogenization pressures and time was not significant ($P > 0.05$) (Table 1), and

Table 5. Protease activity difference[1] of the homogenized cultures of *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12, *Streptococcus salivarius* ssp. *thermophilus* ST-M5, and *Lactobacillus acidophilus* LA-K

Pressure (MPa)	*L. delbrueckii* ssp. *bulgaricus* LB-12	*S. thermophilus* ST-M5	*L. acidophilus* LA-K
0 (control)	0.546	−0.0042	0.062
3.45	0.458	0.022	0.0543
6.9	0.477	0.01	0.0434
10.34	0.492	0.016	0.06
13.8	0.548	0.02	0.052

[1]Protease activity difference = (absorbance value at 12 h – absorbance value at 0 h) obtained (by subtracting the absorbance values at 0 h from h 12) for the same microorganism at the same homogenization pressure.

homogenization pressure did not have a significant ($P > 0.05$) effect on growth of ST-M5 (Table 1). Strain ST-M5 subjected to the different homogenization pressures, including 0 MPa, exhibited a lag phase for 3 h, a logarithmic phase during 3 to 8 h of incubation, and a stationary phase thereafter (Figure 4B).

***L. acidophilus* LA-K.** Growth of LA-K (expressed as log cfu/mL) at different homogenization pressures is shown in Figure 4C. The interaction effect between homogenization pressure and time was not significant ($P > 0.05$) (Table 1), and homogenization pressure did not have a significant ($P > 0.05$) influence on growth (Table 1). In the control, a significant ($P < 0.05$) increase in the viability of LA-K was observed after 3 h of incubation (Figure 4C). In LA-K subjected to 3.45 and 13.80 MPa, a significant ($P < 0.05$) increase in the viable counts was observed after 6 h (Figure 4C). In LA-K subjected to 6.90 and 10.34 MPa, a significant ($P < 0.05$) increase in viable counts was observed after 5 h (Figure 4C). The strain was still in the logarithmic phase after 10 h of incubation for all cultures studied, including the control.

Simova et al. (2006) analyzed the growth profile of *S. thermophilus* T15 and *L. delbrueckii* ssp. *bulgaricus* HP1 inoculated individually in autoclaved reconstituted skim milk and reported that growth reached the exponential phase in the first 5 h and the stationary phase in 8 to 12 h. In the present study, we found that although the growth of the control *S. thermophilus* reached the exponential phase after 3 h, that of *L. delbrueckii* ssp. *bulgaricus* did not reach exponential phase during the 10 h of incubation. The difference in results could be because *L. delbrueckii* ssp. *bulgaricus* used in the present study was a pure frozen culture inoculated into cool (4°C) autoclaved skim milk, whereas the cultures studied by Simova et al. (2006) were preincubated for 5.5 h before inoculation. Another possible reason could be that the homogenization treatment in the present study might have delayed the logarithmic phase of *L. delbrueckii* ssp. *bulgaricus*. Moreover, Shah et al. (2008) reported that *L. delbrueckii* ssp. *bulgaricus* was the most sensitive species among the 3 bacterial cultures, exhibiting the lowest viability when subjected to high pressures of 480 MPa. The results indicated that rate of growth of the bacterium *L. acidophilus* was delayed on increasing the homogenization pressures.

Homogenization pressures of 6.90, 10.34, and 13.80 MPa significantly ($P < 0.05$) decreased growth of *L. delbrueckii* ssp. *bulgaricus* (Table 3), but homogenization pressures did not have significant ($P > 0.05$) effect on growth of *L. acidophilus* and *S. thermophilus* (Tables 1 and 3). *Lactobacillus acidophilus* exhibited a significant ($P < 0.05$) increase in viable counts after 6 h but did not reach stationary phase during 10 h of incubation (Figure 4 C). This is in accordance with results of Liong and Shah (2005), who stated that growth of *L. acidophilus* was predominant in the first 9 to 15 h, after which it reached a stationary phase. Differences in the viable counts of cultures homogenized at different pressures obtained by subtracting viable log cfu/mL counts at 0 h from those at 10 h of incubation are reported in Table 4. In Table 4, a positive number indicates bacterial growth, a negative number indicates bacterial death; a higher positive number means a higher growth rate of the culture and greater resistance to low pressure homogenization. Among the 3 bacterial cultures, *L. delbrueckii* ssp. *bulgaricus* was least resistant, whereas *S. thermophilus* exhibited the highest resistance to low homogenization pressures (Table 4). Similar observations were noted by Shah et al. (2008), who subjected cultures to very high pressures using an ultra-high-pressure press and studied culture growth at a single time point after high-pressure treatment. They reported that *S. thermophilus* was the most resistant species, whereas *L. delbrueckii* ssp. *bulgaricus* was the least resistant when subjected to a pressure of 480 MPa (Shah et al., 2008).

CONCLUSIONS

Acid tolerance of the yogurt cultures *L. delbrueckii* ssp. *bulgaricus* LB-12 and *S. salivarius* ssp. *thermophi-*

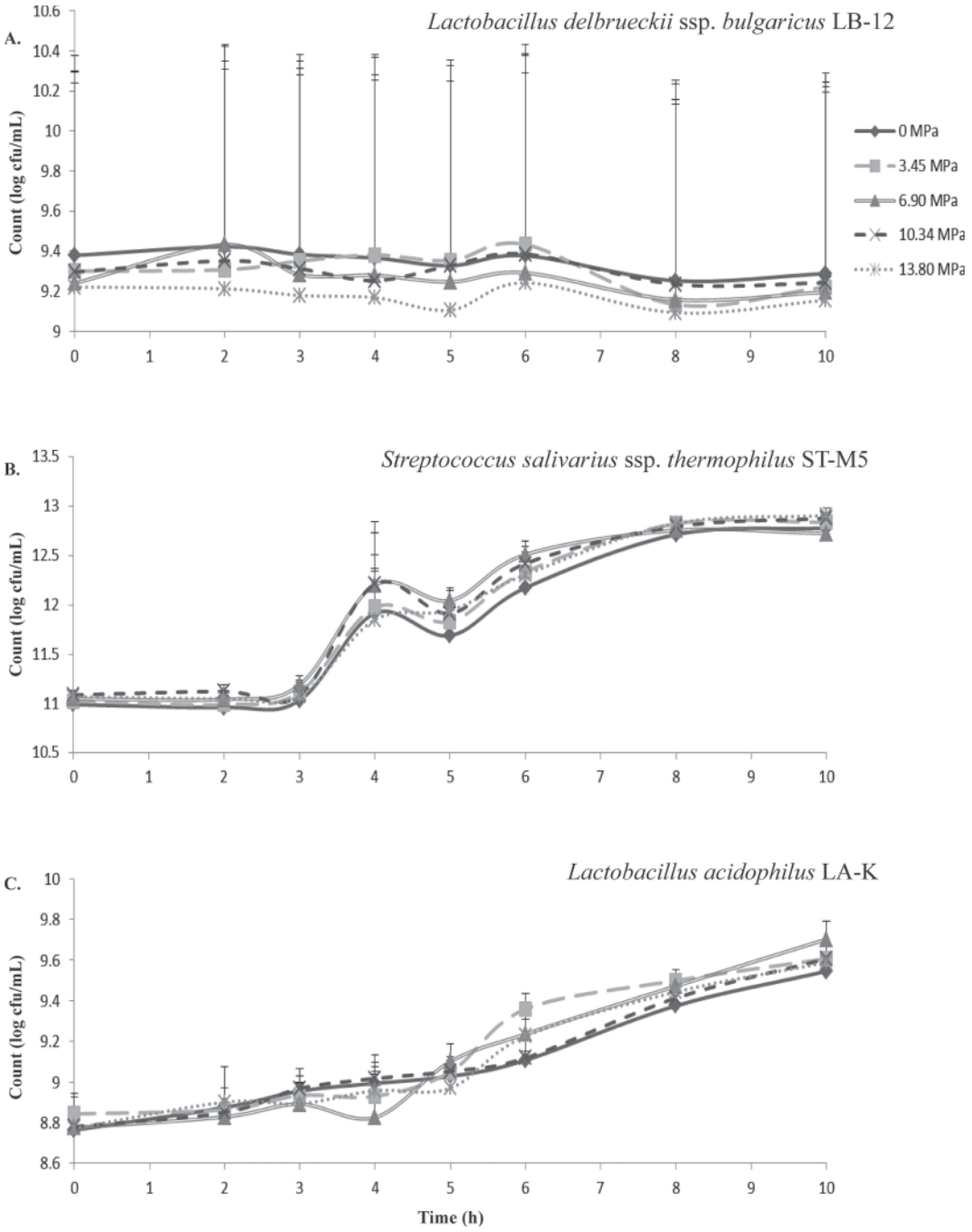

Figure 4. Growth of homogenized cultures of *Lactobacillus delbrueckii* ssp. *bulgaricus* LB-12, *Streptococcus salivarius* ssp. *thermophilus* ST-M5, and *Lactobacillus acidophilus* LA-K at different pressures.

lus ST-M5 was significantly improved by all of the low homogenization pressures under study. Homogenization pressures of 10.34 and 13.80 MPa significantly improved the acid tolerance of *L. acidophilus* LA-K. Bile tolerance of ST-M5 was significantly improved by all low homogenization pressures under study, whereas homogenization pressure of 6.90 MPa significantly improved the bile tolerance of LA-K. The low homogenization pressures under study significantly decreased bile tolerance of LB-12. Protease activity of ST-M5 was significantly increased by the homogenization pressures of 6.90 and 10.34 MPa. Low homogenization pressures did not significantly improve the protease activity of LA-K and LB-12. Growth of LA-K and ST-M5 was

not significantly influenced by homogenization pressure, but growth of LB-12 was significantly decreased by subjecting the culture to various low homogenization pressures. Different bacteria sometimes exhibited a different response to the same probiotic attribute when subjected to the same low homogenization pressure. Some low homogenization pressures positively influenced some characteristics of yogurt culture bacteria and LA-K. Depending upon the improvement in the characteristics of a culture bacterium desired, low homogenization pressure could be used selectively. Culture pretreatment with some low homogenization pressures can be recommended for improvement of certain probiotic characteristics.

ACKNOWLEDGMENTS

The authors thank Jing Wang (Department of Experimental Statistics, Louisiana State University, Baton Rouge) for the statistical consultation.

REFERENCES

Bevilacqua, A., C. Costa, M. R. Corbo, and M. Sinigaglia. 2009. Effects of high pressure of homogenization on some spoilage microorganisms, representative of fruit juice microflora, inoculated in saline solution. Lett. Appl. Microbiol. 48:261–267.

Brinez, W. J., A. X. Roig-Saugués, M. M. Hernándezherrero, and B. Guamislópez. 2006. Inactivation of *Listeria innocua* in milk and orange juice by ultra high pressure homogenization. J. Food Prot. 69:86–92.

Champagne, C. P., Y. Raymond, J. Gonthier, and P. Audet. 2009. Enumeration of the contaminating bacterial microbiota in unfermented pasteurized milks enriched with probiotic bacteria. Can. J. Microbiol. 55:410–418.

Chandan, R. C. 1999. Enhancing market value of milk by adding cultures. J. Dairy Sci. 82:2245–2256.

Coskun, H. 2006. The effect of low pressure-homogenized lactic cultures on the development of proteolysis in cheese slurry. World J. Microbiol. Biotechnol. 22:141–145.

Dairy Facts. 2009. International Dairy Foods Association, Washington, DC.

Dave, R. I., and N. P. Shah. 1996. Evaluation of media for selective enumeration of *Streptococcus thermophilus, Lactobacillus delbrueckii* ssp. *bulgaricus, Lactobacillus acidophilus,* and Bifidobacteria. J. Dairy Sci. 79:1529–1536.

Deshimaru, J. 1994. A study of cavitation in oils: Effect of base oils and polymers. Jpn. J. Tribology 36:531–542.

DHHS-FDA. 2009. 21CFR131.20. Requirements for Specific Standardized Milk and Cream: Yogurt. Accessed May 5, 2001. http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=131.200.

Diels, A. M. J., and C. W. Michiels. 2006. High pressure homogenization as a non-thermal technique for the inactivation of microorganisms. Crit. Rev. Microbiol. 32:201–216.

Donsì, F., G. Ferraria, E. Lenzab, and P. Marescaa. 2009. Main factors regulating microbial inactivation by high-pressure homogenization: Operating parameters and scale of operation. Chem. Eng. Sci. 64:520–532.

Doulah, M. S., and T. H. Hammond. 1975. A hydrodynamic mechanism for the disintegration of *Saccharomyces cerevesiae* in an industrial homogenizer. Biotechnol. Bioeng. 17:845–858.

Dunne, C., L. O. Mahony, L. Murphy, G. Thorton, D. Morrissey, S. O. Halloran, M. Feeney, S. Flynn, G. Fitzgerald, C. Daly, B. Kiely, G. C. O'Sullivan, F. Shanahan, and J. K. Collins. 2001. In vitro selection criteria for probiotic bacteria of human origin: Correlation with in vivo findings. Am. J. Clin. Nutr. 73:386S–392S.

Floury, J., A. Desrumaux, and J. Lardières. 2000. Effect of high-pressure homogenization on droplet size distributions and rheological properties of model oil-in-water emulsions. Innov. Food Sci. Emerg. Technol. 1:127–134.

Gardiner, G. E., E. O'Sullivan, J. Kelly, M. A. E. Auty, G. F. Fitzgerald, and J. K. Collins. 2000. Comparative survival rates of human-derived probiotic *Lactobacillus paracasei* and *L. salivarius* strains during heat treatment and spray drying. Appl. Environ. Microbiol. 66:2605–2612.

Gatti, M., M. E. Fornasari, C. Lazzi, G. Muccheti, and E. Neviani. 2004. Peptidase activity in various species of dairy thermophilic lactobacilli. J. Appl. Microbiol. 96:223–229.

Granato, D., G. F. Branco, A. G. Cruz, J. A. F. Faria, and N. P. Shah. 2010. Probiotic dairy products as functional foods. Compr. Rev. Food Sci. Food Safety 9:455–470.

Guarner, F., G. Perdigon, G. Corthier, S. Salminen, B. Koletzko, and L. Morelli. 2005. Should yogurt cultures be considered probiotics. Br. J. Nutr. 93:783–786.

Heller, L. 2006. Probiotics and health to drive US yogurt market. Accessed Aug. 12, 2008. http://www.foodnavigator-usa.com/Financial-Industry/Probiotics-and-health-to-drive-US-yogurt-market.

Hutkins, R. 2006. Cultured dairy products. Pages 107–114 in Microbiology and Technology of Fermented Foods. Blackwell Publishing, Ames, Iowa.

Kheadr, E., J. F. Vachon, P. Paquin, and I. Fliss. 2002. Effect of dynamic high pressure on microbiological, rheological and microstructural quality of Cheddar cheese. Int. Dairy J. 12:435–446.

Kleinig, A. R., and A. P. J. Middelberg. 1996. Correlation of cell disruption with homogenizer valve pressure gradient determined by computational fluid dynamics. Chem. Eng. Sci. 51:5103–5110.

Lanciotti, R., F. Gardini, M. Sinigaglia, and M. E. Guerzoni. 1996. Effects of growth conditions on the resistance of some pathogenic and spoilage species to high pressure homogenization. Lett. Appl. Microbiol. 22:165–168.

Lanciotti, R., F. Patrignani, L. Iucci, P. Saracino, and M. E. Guerzoni. 2007. Potential of high pressure homogenization in the control and enhancement of proteolytic and fermentative activities of some *Lactobacillus* species. Food Chem. 102:542–550.

Lankaputhra, W. E. V., and N. P. Shah. 1995. Survival of *Lactobacillus acidophilus* and *Bifidobacterium* ssp. in the presence of acid and bile salts. Cult. Dairy Prod. J. 30:2–7.

Lin, M. Y., and C. M. Young. 2000. Folate levels in cultures of lactic acid bacteria. Int. Dairy J. 10:409–413.

Liong, M. T., and N. P. Shah. 2005. Acid and bile tolerance and cholesterol removal ability of lactobacilli strains. J. Dairy Sci. 88:55–66.

Middelberg, A. P. J. 1995. Process scale disruption of microorganisms. Biotechnol. Adv. 13:491–551.

Mohammadi, R., and A. M. Mortazavian. 2011. Review: Technological aspects of prebiotics in probiotic fermented milks. Food Rev. Int. 27:192–212. doi:10.1080/87559129.2010.535235.

Oberg, C. J., B. C. Weimer, L. V. Moyes, R. J. Brown, and G. H. Richardson. 1991. Proteolytic characterization of *Lactobacillus delbrueckii* ssp. *bulgaricus* strains by the *o*-phthaldialdehyde test and amino acid analysis. J. Dairy Sci. 74:398–403.

Pandolfe, W. D. 1982. Development of new gaulin micro gap homogenizing valve. J. Dairy Sci. 65:2035–2044.

Pereira, D. I. A., and G. R. Gibson. 2002. Cholesterol assimilation by lactic acid bacteria and bifidobacteria isolated from the human gut. Appl. Environ. Microbiol. 68:4689–4693.

Prasad, J., P. McJarrow, and P. Gopal. 2003. Heat and osmotic stress responses of probiotic *Lactobacillus rhamnosus* HN001 (DR20) in relation to viability after drying. Appl. Environ. Microbiol. 69:917–925.

Sandholm, M. T., P. Myllarinen, R. Crittenden, G. Mogensen, R. Fondlen, and M. Saarela. 2002. Technological challenges for future probiotic foods. Int. Dairy J. 12:173–182.

SAS Institute. 2003. SAS Software 9.1 for Windows. SAS Institute Inc., Cary, NC.

Scholz-Ahrens, K. E., P. Ade, B. Marten, P. Weber, W. Timm, Y. Ail, C. C. Glüer, and J. Schrezenmeir. 2007. Prebiotics, probiotics, and symbiotic affect mineral absorption, bone mineral content, and bone structure. J. Nutr. 137:838S–846S.

Shah, N. P., and P. Jelen. 1990. Survival of lactic acid bacteria and their lactases under acidic conditions. J. Food Sci. 55:506–509.

Shah, N. P., D. Tsangalis, and O. N. Donkor. 2008. Effect of high pressure treatment on viability of *Lactobacillus delbrueckii ssp. bulgaricus, Streptococcus thermophilus* and *L. acidophilus* and the pH of fermented milk. Milchwissenschaft 63:11–14.

Simova, E., Z. Simov, D. Beshkova, G. Frengova, Z. Dimitrov, and Z. Spasov. 2006. Amino acid profiles of lactic acid bacteria, isolated from kefir grains and kefir starter made from them. Int. J. Food Microbiol. 107:112–123.

Soda, M. E. 1991. Recent developments in accelerated cheese ripening. J. Dairy Sci. 74:2317–2335.

Talwalkar, A., and K. Kailasapathy. 2003. Effect of microencapsulation on oxygen toxicity in probiotic bacteria. Aust. J. Dairy Technol. 58:36–39.

Tuomola, E., R. Crittenden, M. Playne, E. Isolauri, and S. Salminen. 2001. Quality assurance criteria for probiotic bacteria. Am. J. Clin. Nutr. 73:393S–398S.

Vachon, J. F., E. E. Kheadr, J. Giasson, P. Paquin, and I. Fliss. 2002. Inactivation of some food pathogens in milk using dynamic high pressure. J. Food Prot. 65:345–352.

Vannini, L., R. Lanciotti, D. Baldi, and M. E. Guerzoni. 2004. Interactions between high pressure homogenization and antimicrobial activity of lysozyme and lactoperoxidase. Int. J. Food Microbiol. 94:123–135.

Wilkinson, M. G., and K. N. Kilcawley. 2005. Mechanisms of incorporation and release of enzymes into cheese during ripening. Int. Dairy J. 15:817–830.