Short communication: Lactose enhances bile tolerance of yogurt culture bacteria

Behannis Mena* and Kayanush Aryana†¹

*School of Agriculture and Food, Faculty of Veterinary and Agricultural Sciences, The University of Melbourne, Parkville, VIC 3010, Australia

†School of Nutrition and Food Sciences, Louisiana State University Agricultural Center, Baton Rouge 70803

ABSTRACT

Lactose is an energy source for culture bacteria. Bile tolerance is an important probiotic property. Our aim was to elucidate the effect of lactose on bile tolerance of yogurt starter culture *Lactobacillus bulgaricus* LB-12 and *Streptococcus thermophilus* ST-M5. Bile tolerance of pure cultures was determined using 0.3% oxgall in MRS THIO broth (Difco, Becton Dickinson, Sparks, MD) for *L. bulgaricus* and 0.3% oxgall in M17 broth (Oxoid, Basingstoke, UK) for *Strep. thermophilus*. Lactose was added to both broths at 0 (control), 1, 3, and 5% (wt/vol) broth. Dilutions were plated hourly for 12 h. Experiments were replicated 3 times. At 2, 4, and 12 h of incubation, lactose incorporated at all amounts, 1, 3, and 5% (wt/vol), showed higher counts of *Strep. thermophilus* ST-M5 compared with the control. Lactose use at 5% (wt/vol) significantly enhanced bile tolerance of both *L. bulgaricus* and *Strep. thermophilus* compared with control.

Key words: lactose, bile tolerance, yogurt starter culture

Short Communication

Bile is discharged in the small intestine and it destroys the cell membranes of bacteria, lowering their survival (Succi et al., 2005). Major components of the bacterial cell membrane are lipids and fatty acids. Changes in cell membrane components influence cell viability, permeability, and interactions between the environment and bacterial cell membrane (Succi et al., 2005). Tolerance to bile is an essential probiotic attribute. Bile concentration of 0.15 to 0.3% is appropriate for selection of probiotics (Goldin and Gorbach 1992).

Lactose or milk sugar is a major solids component in fluid milk and nonfat dry milk. Yogurt is made from milk fermented using yogurt culture bacteria *Lacto-*

bacillus bulgaricus and *Streptococcus thermophilus*. From outside the bacterial cell, lactose is transferred into the *Strep. thermophilus* cell by membrane-bound β -galactoside permease (Neves et al., 2005). Once inside the bacterial cell, lactose is cleaved by β -galactosidase into glucose and galactose (Venkatesh et al., 1993).

Lactose is an energy source for living organisms. As bile and lactose influence bacterial cell viability in opposite ways, one would question whether lactose would affect bile tolerance of yogurt culture bacteria. To the best of our knowledge, no reports exist on the effect of lactose on yogurt culture bacterial response to bile. The aim of the current study was to determine the influence of various concentrations of lactose on the bile tolerance of the yogurt starter culture.

Bile tolerance was determined according to Dave and Shah (1996) and Pereira and Gibson (2002) with minor changes. Sodium thioglycolate (Acros Organics, Fair Lawn, NJ) 0.2% (wt/vol) was used as oxygen scavenger to make THIO de Man, Rogosa, Sharpe (MRS) broth (Difco, Becton Dickinson, Sparks, MD). The THIO MRS broth was prepared for *L. bulgaricus*. The M17 broth (Oxoid, Basingstoke, UK) was prepared for *Strep. thermophilus*. The THIO MRS and M17 broths were supplemented individually with 0.3% (wt/vol) oxgall (bovine bile; US Biological, Swampscott, MA). Lactose was incorporated into both broths at 0 (control), 1, 3, and 5% (wt/vol). Both broths were inoculated individually with 1% (vol/vol) of freshly thawed pure culture *L. bulgaricus* LB-12 and *Strep. thermophilus* ST-M5 (Chr. Hansen, Milwaukee, WI). Inoculated broths were incubated aerobically for 12 h at 37°C for *Strep. thermophilus* ST-M5 and anaerobically at 43°C for *L. bulgaricus* LB-12.

Serial dilutions of inoculated broths were conducted hourly in 0.1% (wt/vol) peptone water. Pour plating was conducted in duplicate. Lactobacilli MRS agar was used for the enumeration of *L. bulgaricus* LB-12 (Dave and Shah, 1997) and M17 agar was used for the enumeration of *Strep. thermophilus* ST-M5 (Jordano et al., 1992). The *L. bulgaricus* LB-12 petri dishes were anaerobically incubated for 72 h at 43°C. The *Strep.*

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¹Corresponding author: karyana@agcenter.lsu.edu

Figure 1. Bile tolerance of *Streptococcus thermophilus* ST-M5 as influenced by the addition of 0 (control), 1, 3, or 5% lactose over the incubation period of 12 h. Data are expressed as means $\pm$ SE.

thermophilus ST-M5 petri dishes were aerobically incubated for 24 h at 37°C. The colonies were counted after the incubation period. All experiments were replicated 3 times.

Bacterial culture counts were converted to $\log_{10}$ scale before data analyses; Proc Mixed of the SAS (version 9.3, SAS Institute Inc., Cary, NC) was used to analyze data. Differences between least square means were determined at $\alpha = 0.05$ for the main effects (lactose concentration and time) and interaction effects (lactose concentration $\times$ time).

The bile tolerance of *Strep. thermophilus* ST-M5 as affected by lactose addition over the incubation of 12 h is presented in Figure 1. We noted a significant ($P < 0.05$) interaction effect for lactose concentration $\times$ time. The main effects, namely lactose concentration effect and time (h), were also significant ($P < 0.05$). At h 2, 4, and 12 of incubation, lactose incorporated at 1, 3, and 5% (wt/vol) showed significantly ($P < 0.05$) higher counts in comparison to control. Gilliland and Kim (1983) found that the use of 1 and 0.5% oxgall enhanced ($P < 0.01$) the lactose-hydrolyzing activity of yogurt starter bacteria to 16.7 and 19.8 units, respectively. Martini et al. (1987) observed a 3-fold increase in lactase activity of yogurt starter culture with use of 0.5 or 1% oxgall and mentioned that bacterial cell permeability was altered when exposed to intestinal bile.

Streptococcus thermophilus ST-M5 counts at 0 h were subtracted from those at 12 h of incubation to obtain the mean log difference in the viable counts. A large number indicated higher bacterial survival. Bacterial survival was greatest (0.59 log cfu/mL) for 5% (wt/vol) lactose compared with the remaining (i.e., 0.40, 0.18, and 0.08 log cfu/mL for 3, 1, and 0% lactose, respectively). By increasing energy production through sugar metabolism, responses against damaging action of bile at various levels, such as fatty acid biosynthesis, bile efflux, and cell wall design, can be accomplished in lactic acid bacterial strains (Ruiz et al., 2013).

Bile tolerance of *L. bulgaricus* LB-12 as affected by addition of lactose over 12 h of incubation is shown in Figure 2. The interaction effect of lactose concentration $\times$ time was not significant ($P > 0.05$). The main effects, that is, lactose concentration effect and time (h), were significant ($P < 0.05$). Use of 5% (wt/vol) lactose resulted in higher ($P < 0.05$) counts compared with use of 1% (wt/vol) lactose or no lactose (control). The highest counts of *L. bulgaricus* LB-12 were observed during first 8 h of incubation. *Lactobacillus bulgaricus* LB-12 log colony-forming units per milliliter at 12 h of incubation were subtracted from counts at 0 h to obtain the mean log difference in viable counts. A smaller number indicated lower bacterial death. Bacterial death was the least (0.92 log cfu/mL) for 5% (wt/vol) lactose

Figure 2. Bile tolerance of *Lactobacillus bulgaricus* LB-12 as influenced by the addition of 0 (control), 1, 3, or 5% lactose over the incubation period of 12 h. Data are expressed as means $\pm$ SE.

compared with the remaining (i.e., 1.25, 1.33, and 1.29 log cfu/mL for 3, 1, and 0% lactose, respectively).

Lactose had a beneficial influence on bile tolerance of yogurt starter bacteria. From a manufacturing standpoint, because nonfat dry milk and whey powders contain lactose they also can be potential ingredient options to improve bile tolerances of yogurt culture bacteria.

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