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Effect of Lactose on Acid Tolerance of Yogurt Culture Bacteria

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Abstract

Lactose, the sugar naturally present in milk, provides energy to lactic acid bacteria used in fermented dairy foods. Increasing concentrations of lactose may improve survivability of lactic acid bacteria in the dairy foods and in human gut enhancing their probiotic benefits. Acid tolerance is an important probiotic characteristic. The objective was to determine the influence of lactose on acid tolerance of yogurt starter culture *Streptococcus thermophilus* ST-M5 and *Lactobacillus bulgaricus* LB-12. The M 17 broth was used for *Streptococcus thermophilus* ST-M5 and MRS broth was used for *Lactobacillus bulgaricus* LB-12. Lactose was added to both broths at 0% (control), 1%, 3%, and 5% (wt/vol). Both broths were acidified to pH 2.0. Upon sterilizing and tempering, both broths were inoculated. Acid tolerance was determined as viable counts in acidified broths after 120 minutes of incubations. In an incubation period of 2 hours, dilutions were plated every 30 minutes. Three replications were conducted. The highest acid tolerance for *Streptococcus thermophilus* ST-M5 and *Lactobacillus bulgaricus* LB-12, was observed in lactose concentration of 3% and 5% (wt/vol).

Keywords

Lactose, Acid Tolerance, Lactic Acid Bacteria

1. Introduction

Lactic acid bacteria (LAB) must survive the adverse conditions, such as an acidic environment of the stomach, prior to establishing in the lower gastrointestinal (GI) tract before conferring health benefits upon the human host. Tolerance to acid is an important probiotic trait, which determines LAB survival and growth [1]

[2]. The LAB genera are typically made up of low proteolytic activity-fermentative strains, which means that they ingest and metabolize sugars to produce essentially lactic and acetic acids as their catabolic products.

The pH of the stomach, generally, ranges from 2.0 to 4.0 [3]. Berrada *et al.* [4] reported that it takes 90 minutes for the bacteria to be released after the time from entrance into the stomach. Therefore, strains selected to be used as probiotic bacteria should be able to tolerate acid for at least 90 minutes, attach to the epithelium, and grow in the lower intestinal tract before they can start providing health benefits [5]. Standards for acid tolerance of probiotic culture have been established as surviving pH of 2.0 for 2 hours [6].

Lactose is a disaccharide carbohydrate, composed of two monosaccharide components: D-glucose and D-galactose [7]. This carbohydrate provides a ready source of energy to living organisms. Lactose is not metabolized directly by LAB. This carbohydrate is transferred into the bacterial cell where it is hydrolyzed to glucose and galactose by the lactose-permease enzymes [8]. The release of β-galactosidase from bacterial cells during the transit through the small intestine, appears to be linked to the mechanism of lactose digestion. Yogurt culture strains have the ability to hydrolyze lactose; thereby, yogurt cultures function as a source of enzymes in the intestinal tract [9].

Streptococcus thermophilus and *Lactobacillus delbrueckii* ssp. *bulgaricus*, are able to produce extracellular polysaccharides (EPS) that exert one of two functions: encapsulate the bacteria or are excreted into the extracellular environment [10]. This is considered as a natural encapsulation for the bacteria which protects them from adverse environmental conditions such as low pH.

Authors wondered whether lactose would have a positive effect on acid tolerance of yogurt starter culture. To our knowledge, studies related to the effect of lactose on the acid tolerance of yogurt starter bacteria are scarce. The relationship that exists between several lactose concentrations and the bacterial survival is unknown. The aim of this study was to determine the influence of various concentrations of lactose on acid tolerance of yogurt starter culture *Streptococcus thermophilus* ST-M5 and *Lactobacillus bulgaricus* LB-12.

2. Materials and Methods

Acid tolerance was determined according to the method proposed by Pereira and Gibson [11], with slight modifications. Broths were acidified to pH 2 using 1 N HCL and lactose was incorporated at 0% (control), 1%, 3%, and 5% (wt/vol). Freshly thawed pure culture *L. bulgaricus* LB-12 and *Streptococcus thermophilus* ST-M5 (Chr. Hansen, Milwaukee, WI) were inoculated separately with 1% (vol/vol) into control and lactose broths. For *Lactobacillus bulgaricus* LB-12, MRS broth (DifcoTM, Dickinson and company, Sparks, MD) was prepared and M17 broth (Oxoid, Basingstoke, UK) was prepared for *Streptococcus thermophilus* ST-M5. Inoculated broths were incubated anaerobically at 43˚C for *Lactobacillus bulgaricus* LB-12 and aerobically at 37˚C for *Streptococcus thermophilus* ST-M5 for 2 hours. Peptone water (0.1% wt/vol) was used to serially dilute an aliquot of the inoculated broths which were subsequently pour plated in duplicate every 30 minutes up to 2 hours. *Lactobacillus bulgaricus* LB-12 was enumerated using *Lactobacilli* MRS agar [12] and M17 agar was used for enumeration of *Streptococcus thermophilus* ST-M5 [13]. Petri dishes were incubated anaerobically at 43˚C for 72 hours for *Lactobacillus bulgaricus* LB-12 and aerobically at 37˚C for 24 hours for *Streptococcus thermophilus* ST-M5. A colony counter (Darkfield Quebec Colony Counter, American Optical, Buffalo, NY) was used, after the incubation period, to assist the enumeration of colonies. Three replications were performed.

Bacterial counts were converted to $\log_{10}$ scale before analyzing the data with Proc Mixed SAS (version 9.3, SAS Institute Inc., Cary, NC). Significant differences between least square means were determined at $\alpha = 0.05$ for main effects (lactose concentration and time) and interaction effects (lactose concentration × time) using Tukey adjustment.

3. Results and Discussion

The acid tolerance of *Streptococcus thermophilus* ST-M5 as influenced by lactose addition over incubation of 120 minutes is shown in **Figure 1**. A significant ($P < 0.05$) interaction effect for lactose concentration × time was noted. The main effects, namely lactose concentration and time (minutes), were also significant ($P < 0.05$). Treatments 3% and 5% (wt/vol) lactose showed significantly higher viable cell counts compared to control and 1% (wt/vol) lactose at 0 and 120 minutes.

By subtracting log CFU/mL of 120 minutes from 0 minutes of incubation, mean log difference in the counts of *Streptococcus thermophilus* ST-M5 was obtained (**Table 1**). Lower bacterial death is indicated by a low number. The lowest

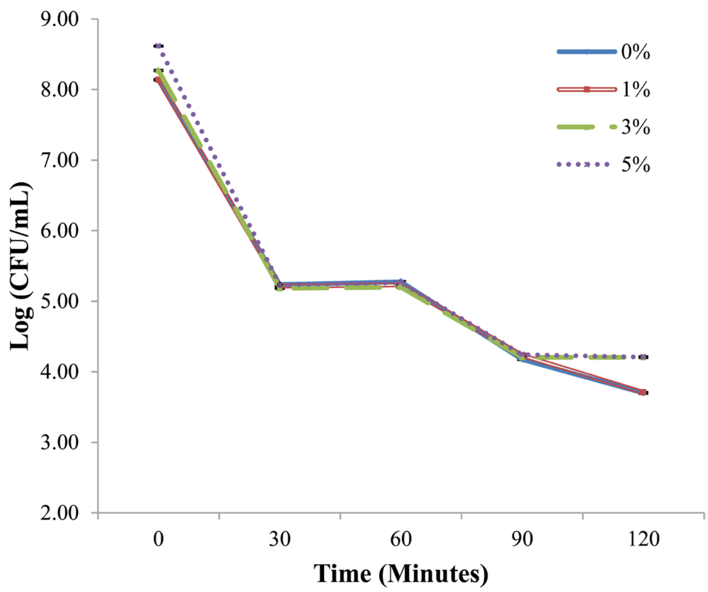

Figure 1. Influence of lactose concentration on acid tolerance of *Streptococcus thermophilus* ST-M5 over the incubation period of 120 minutes.

bacterial death was observed for 3% (wt/vol) lactose compared to the rest. According to Van de Guchte *et al.* [14], the LAB including *Streptococcus thermophilus*, grow generally between pH 5 and 9. *Streptococci* ssp. are susceptible to low pH. Exposure of *Streptococcus macedonicus* to pH 3.5 for 45 minutes resulted in almost 100% death as reported by Papadimitriou *et al.* [15].

Acid tolerance of *Lactobacillus bulgaricus* LB-12 as influenced by lactose addition over incubation of 120 minutes is shown in **Figure 2**. Main effects, lactose concentration and time (minutes) were significant ($P < 0.05$). The interaction effect of lactose concentration × time was also significant ($P < 0.05$). Treatments 3% and 5% (wt/vol) lactose had a significantly higher counts ($P < 0.05$) compared to control from 90 minutes of incubation. The 5% (wt/vol) lactose showed significantly the highest viable cell counts compared to control at 30, 90 and 120 minutes of incubation.

Mean log difference in the viable counts of *Lactobacillus bulgaricus* LB-12 (**Table 1**) was obtained as described above. The lowest bacterial death was observed for

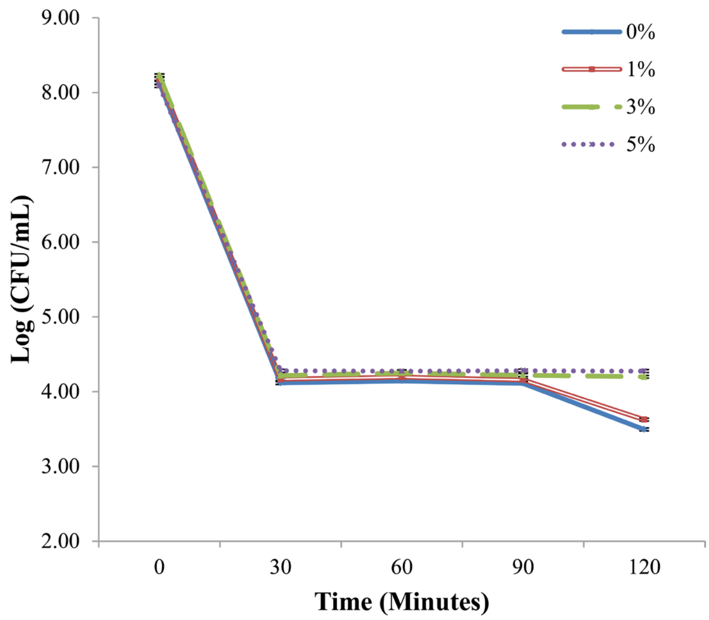

Figure 2. Influence of lactose concentration on acid tolerance of *Lactobacillus bulgaricus* LB-12 over the incubation period of 120 minutes.

Table 1. Influence of lactose concentrations in the presence of acid on mean log difference in the viable counts (Log CFU/mL) of *Streptococcus thermophilus* ST-M5 and *Lactobacillus bulgaricus* LB-12.

Added Lactose Concentration (%)	*Streptococcus thermophilus* ST-M5	*Lactobacillus bulgaricus* LB-12
Control	4.44	4.64
One	4.43	4.56
Three	4.06	4.04
Five	4.41	3.81

5% (wt/vol) lactose compared to the rest. *Lactobacillus bulgaricus* is known to be more acid tolerant compared to *Streptococcus thermophilus*. Shah and Jelen [16] while studying the survival of lactic acid bacteria and their lactases under acidic conditions reported that both *Lactobacillus delbrueckii* ssp. *bulgaricus* and *Streptococcus thermophilus* survived pH as low as 1.5.

4. Conclusion

Higher lactose concentrations had a positive influence on acid tolerance of yogurt culture bacteria. This could also represent a potential way to improve the survivability of both these LAB and hence their probiotic benefits in the human gut.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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