SURVIVAL OF CELLS OF *LACTOBACILLUS ACIDOPHILUS* AND *LACTOBACILLUS CASEI* DURING REFRIGERATED STORAGE IN FERMENTED MILK PRODUCTS

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Story in Brief

Viability of *Lactobacillus acidophilus* and *Lactobacillus casei* added as adjuncts in yogurt and cultured buttermilk during 28 days of refrigerated storage was investigated using five different strains of *L. acidophilus* and one strain of *L. casei.* Colonies were enumerated on LBS and LBS-O agars. At each sampling period, colonies from the selective agar medium were isolated for characterization and comparison using a commercially available identification kit (API CH 50). This helped ensure that the strains of *L. acidophilus* or *L. casei* and not the traditional buttermilk or yogurt cultures were recovered. Generally, *L. acidophilus* survived better in cultured buttermilk than in yogurt. However, strains of *L. acidophilus* differed in survival in both cultured products. *L. casei* survived very well in both cultured products.

(Key Words: *Lactobacillus acidophilus, Lactobacillus casei*).

Introduction

Health benefits associated with fermented milk products can be provided by the bacterial starter culture or by dietary adjuncts added after the product is fermented. Improved digestion of lactose, aid in control of serum cholesterol levels, antagonistic action toward pathogens and control of certain types of intestinal cancer are the primary potential health benefits associated with fermented products containing *Lactobacillus acidophilus* and *Lactobacillus casei* as dietary adjuncts.

L. acidophilus and *L. casei* can be added easily to fermented products such as buttermilk or yogurt. However, for most of the potential health benefits to the consumer, it is necessary that the cells be viable when consumed. Typically, these products are consumed within 2 to 3 weeks after production. Therefore, the cells should survive for at least 3 weeks to provide maximum health benefits to the consumers. The objective of this study was to determine the effect of refrigerated storage in both buttermilk and yogurt on five strains of *L. acidophilus* and one strain of *L. casei* during a 28 day storage period.

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Materials and Methods

Two strains of *L. acidophilus*, La-5 and MUH-41, and the one of *L. casei* were obtained from the Mona Division of Campina Melkunie; the other three *L. acidophilus* strains ATCC 43121, L-1 and O-16, were obtained from the stock culture collection in our lab. The yogurt cultures, CM-2 and YC-4, and the buttermilk culture also were obtained from Campina Melkunie.

MRS broth was used for propagation of the lactobacilli. For each culture, 20 ml of MRS broth was inoculated at 1% using a freshly prepared culture of the desired strain of lactobacilli and incubated at 37°C for 18 hours. Cells were removed by centrifugation and resuspended in 10 ml of sterile, reconstituted 10% nonfat dry milk (NDM). This cell suspension containing approximately 9 x 10^8 cells/ml was held in ice water until it was added to the yogurt or buttermilk.

Buttermilk was prepared from raw milk standardized to contain 0.5% butterfat. The milk was homogenized, pasteurized, and then cooled to 21°C before it was inoculated at 1% with the buttermilk culture. The inoculated milk was dispensed in 900 ml portions into eight sterile bottles. The bottles were incubated at 21°C until a pH of 4.55 was reached. The buttermilk was cooled and the appropriate amount of the cell suspensions of the desired lactobacilli were added to yield a population of approximately 1 x 10^7 cfu/ml. The 900 ml portions of the buttermilk were each mixed thoroughly and dispensed into five sterile dilution bottles and stored at 5°C. A control containing no supplemental lactobacilli was treated in the same manner. One bottle for each adjunct culture was evaluated for total and bile tolerant supplemental lactobacilli on days 0, 7, 14, 21 and 28.

Yogurt was prepared from raw milk standardized to contain 3% milkfat. The raw milk was supplemented with an additional 3.5% NDM. The mixture was homogenized, pasteurized and tempered to 45°C. It then was inoculated with 2% of the desired yogurt culture and incubated until a pH of 4.9 was reached. The yogurt was cooled and dispensed in 1000 g quantities into sterile beakers. Appropriate amounts of cell suspensions of the lactobacilli were added to yield an initial population of approximately 1 x 10^7 cfu/g. After mixing the yogurt samples were distributed in 200 g quantities into five plastic cups and stored at 7°C. A sample of each was removed on days 0, 7, 14, 21 and 28 for analyses. A control containing no lactobacilli also was evaluated for total and bile tolerant lactobacilli and pH.

The selective enumeration of the lactobacilli was carried out on modified LBS agar which contained cellibiose in place of glucose as the sugar source. This agar was called C-LBS. Bile-tolerant lactobacilli were enumerated on C-LBSO agar which was C-LBS agar supplemented with 0.1% oxgall. Plates were incubated at 37°C for 48 hours in a CO_2 enriched atmosphere.

Two colonies from the highest dilution plated on C-LBS agar were grown in MRS broth at 37°C for 48 hours for confirmation of the identity of the *L. acidophilus* and the *L. casei*. The API CHL 50 identification system was used to test the action of the cultures on 50 substrates. The cultures also were evaluated for their ability to grow at 15 and 45°C and their Gram stain reaction.

Results and Discussion

Total numbers of *L. acidophilus* 43121 declined significantly ($P<.05$) with increased storage time at 5°C in buttermilk (Table 1). The decline became significant ($P<.05$) on day 21 and there was a decline of approximately 1 log by day 28. There was a slight, although insignificant ($P>.05$), increase in numbers from day 0 to 7. This trend was observed for practically all strains in both buttermilk and yogurt and in some cases it became significant. This can be attributed to the breaking up of clumps or chains of the supplemental lactobacilli.

L. acidophilus strain La-5 decreased significantly ($P<.05$) in total numbers after 28 days of storage in the buttermilk (Table 1).

There were no significant ($P>.05$) declines in total numbers compared with initial counts during the 28 day storage period for strains MUH-41, O-16, L-1 and *L. casei* (Table 1).

Declines in bile tolerant lactobacilli were similar to those observed for total lactobacilli. However, there generally were lower counts on C-LBSO agar than on C-LBS agar (Table 1). For each of *L. acidophilus* 43121, MUH-41, and La-5, there was a significant ($P<.05$) decline in numbers of bile tolerant lactobacilli after 14 days of storage in the buttermilk. There were significantly ($P<.05$) lower counts on C-LBSO agar than on C-LBS agar on days 14, 21, and 28 for strain 43121 and on days 7, 14, 21 and 28 for strain MUH-41. Strain La-5 differed in the two counts on days 14 and 21.

L. acidophilus O-16, L-1 and the *L. casei* showed no decline in numbers of bile tolerant lactobacilli during the 28-day storage period and there were no differences between counts on C-LBS and C-LBSO agars (Table 1).

There were no significant ($P>.05$) differences between the total number and number of bile-tolerant lactobacilli for any strain of *L. acidophilus* or *L. casei* on any day of sampling for yogurt made with culture CM-2 (Table 2).

Total numbers of *L. acidophilus* 43121 had declined significantly ($P<.05$) by day 28. Numbers of strains MUH-41, La-5, and O-16 had declined significantly ($P<.05$) after 21 days of storage (Table 2). Total numbers of *L. acidophilus* L-1 and *L. casei* did not decline significantly ($P>.05$) over the entire 28-day storage period.

The pH values decreased over time from about 4.5 to 4.3 by day 28 depending on the strain. This was the trend observed in the control yogurt also so the supplemental lactobacilli probably did not affect the pH.

The behavior of the supplemental lactobacilli was different in yogurt made with the YC-4 yogurt starter culture. The total numbers of *L. acidophilus* 43121 and *L. casei* did not decline significantly (P>.05) during the entire 28 day storage period (Table 3). However, the other strains exhibited significant (P<.05) declines. Strain MUH-41 had declined significantly (P<.05) by day 28 compared to initial counts. Numbers of O-16 had declined significantly (P<.05) by day 14 and after.

The total numbers of *L. acidophilus* L-1 were stable until day 28 when they exhibited a significant (P<.05) decline (Table 3). Strain La-5 had declined significantly (P<.05) in total number after 14 days of storage and after.

Counts of bile-tolerant lactobacilli for strains 43121, L-1, and *L. casei* were similar to total numbers. However, the other strains differed somewhat. Strain MUH-41 had similar declines for both counts, but numbers were significantly (P<.05) higher on C-LBSO compared with C-LBS on days 7, 21, and 28 (Table 3). The declines in numbers of bile tolerant lactobacilli for strains O-16, and La-5 were significant (P<.05) on day 14 and again on day 28. There were significantly (P<.05) higher counts on C-LBSO compared with C-LBS on days 21 and 28 for strain 0-16. For strain La-5 where were differences in counts of total and bile tolerant lactobacilli on day 21 (Table 3).

The initial pH values of the yogurts were 4.8-5.0 depending on the strain. They declined in a manner similar to the control. Therefore, the supplemental lactobacilli probably did not affect the pH.

All isolates from both buttermilk and yogurt were confirmed to be *L. acidophilus* or *L. casei* using our identification system. There were no colonies formed on plates from the control samples. Thus the CLBS and C-LBSO agars enumerated only the supplemental lactobacilli.

In conclusion, strains of *L. acidophilus* and *L. casei* varied in their ability to remain viable during refrigerated storage in the two fermented milk products in this study. The addition of an appropriate strain of *L. acidophilus* or *L. casei* to cultured buttermilk or yogurt after fomentation at a level of approximately 1 x 10^7 cfu/ml can result in numbers of viable cells in excess of 1 x 10^6 cfu/ml after 28 days of storage at 5 or 7 C. This indicates that fermented products are suitable carriers for these supplemental lactobacilli. Results of this study emphasize the importance of selecting the appropriate strains of *L. acidophilus* or *L. casei* and the appropriate starter culture for preparing a fermented product to provide the optimum potential health benefits to consumers.