

# Viability of Bifidobacteria in Commercial Dairy Products during Refrigerated Storage

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## ABSTRACT

Commercial milk and two brands of yogurt containing bifidobacteria were obtained from retail outlets. All products were evaluated for viability of bifidobacteria and lactic acid bacteria during refrigerated storage at 4°C. Milk was evaluated at 9, 6, and 3 days prior and past its expiration date. The yogurts were evaluated at 3, 2, and 1 week prior and past their expiration. Viability of bifidobacteria and lactic acid bacteria in milk and yogurt remained above 10<sup>6</sup> CFU/ml or g until the expiration date of the respective products. This microbial concentration is the recommended minimum dose to receive the health benefits of these organisms.

The current reported health benefits of bifidobacteria include inhibition of bacterial pathogens, reduction of colon cancer risk, stimulation of the immune response, and reduction of serum cholesterol levels (26, 30). Because bifidobacteria have been associated with health-promoting effects, there has been an increasing interest in incorporating this microbial group into fermented dairy foods or supplementing dairy foods with these organisms. The ultimate intent of this strategy is to provide the gastrointestinal tract of humans with viable populations of bifidobacteria.

For bifidobacteria to provide therapeutic effects, it has been recommended that they be viable and ingested in numbers  $\geq 10^6$  cells/g (13). Thus, maintaining viability of these organisms until the products are consumed in order to ensure the delivery of live organisms has been of much interest. Although scientific opinions regarding the significance of viability in the therapeutic efficacy of lactic acid bacteria and bifidobacteria still remain divided and need further clarification from the scientific community, the public expects fermented dairy products to contain viable organisms at the time of consumption (1). In Japan, the Fermented Milks and Lactic Acid Beverages Association has already established a standard that requires  $\geq 10^7$  viable bifidobacteria/ml to be present in dairy products that claim to contain bifidobacteria (10). The Swiss Food Regulation as well as the International Standard of FIL/IDF require that such products contain  $\geq 10^6$  CFU/g of bifidobacteria.

Studies by Biavati et al. (2), Klaver et al. (12), Shah et al. (28), Kailasapathy and Rybka (11), Dave and Shah (4, 5), and Rybka and Fleet (24) have shown that bifidobacteria grow poorly in milk and do not survive well in the final product. Maintaining viability of bifidobacteria in milk and in fermented dairy foods has been a challenge to the dairy processors because the organism requires low oxidation reduction potential for growth (29) and is sensitive

to low pH. The organism also requires specific growth factors (12, 19, 20, 23). Various researchers have reported on the viability of bifidobacteria in dairy products (25), such as fermented milk (16, 17), creamed cottage cheese (3), frozen fermented desserts (15), ice-cream (9), and yogurt (18, 28).

Today in the U.S. approximately 60% of refrigerated yogurts contain probiotic cultures *Lactobacillus acidophilus* and/or *Bifidobacterium* sp. (1). Although yogurt consumption in the U.S. continues to increase steadily due to the perceived prophylactic and therapeutic properties of live and active cultures present (21), there are no well-designed scientific studies reporting the viability of bifidobacteria in commercial U.S. yogurts. For consumers that do not like fermented dairy foods, unfermented milks containing probiotic cultures have been available commercially for the last 25 years. Initially, these products were produced with the addition of *L. acidophilus*. More recently, products containing *Bifidobacterium* sp. were introduced. Viability of bifidobacteria in these commercial unfermented milks sold in the U.S. also is not well documented. Thus, the purpose of this study was to determine the viability of bifidobacteria in commercial U.S. dairy products, more specifically in yogurt and in milk supplemented with *Bifidobacterium* sp.

## MATERIALS AND METHODS

**Sampling.** Commercial A/B milk (supplemented with *L. acidophilus* and bifidobacteria) and two brands of yogurt (containing bifidobacteria in addition to the traditional yogurt cultures *Streptococcus salivarius* subsp. *thermophilus* and *L. delbrueckii* subsp. *bulgaricus*) were obtained from retail outlets in Michigan and stored at  $4 \pm 0.5^\circ\text{C}$  during the duration of the study. All products claimed viable bifidobacteria and lactic acid bacteria on the label. Milk was evaluated at 3-day intervals up to 9 days prior to its expiration and past its expiration. Yogurts on the other hand were evaluated at 1-week intervals up to 3 weeks prior to their expiration and past their expiration. All samples were aseptically removed from each container and diluted by mixing 1 ml of milk

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or 1 g of yogurt with 99 ml of sterile 0.1% (wt/vol) bactopectone (Difco, Detroit, Mich.), and subsequent serial dilutions were made. Another sample was collected for pH measurements. The pH of the products was monitored at each sampling point.

#### Enumeration of bifidobacteria and lactic acid bacteria.

Bifidobacteria were enumerated using MRSL agar (MRS [de Man Rogosa Sharpe] + 5% [wt/vol] lactose; Difco) (7) containing 5% (vol/vol) filter-sterilized (0.22  $\mu$ m) NPNL antibiotic solution containing 2 g/liter neomycin sulfate (Sigma, St. Louis, Mo.), 4 g/liter paromomycine sulfate (Sigma), 0.3 g/liter nalidixic acid (Sigma), and 60 g/liter lithium chloride (Sigma) (32). The inoculated plates were incubated at 37°C for 48 h using Gas Pak (Becton Dickinson Co., Cockeysville, Md.). Lactic acid bacteria were enumerated using MRSL agar. The inoculated plates were incubated aerobically at 37°C for 48 h. Colonies were counted using a Quebec colony counter (Fisher Scientific, Pittsburgh, Pa.).

**Statistical analysis.** All experiments were replicated three times in a randomized block design. Replicates consisted of three batches of milk or yogurts purchased at three different times that were obtained from different lots. One brand of milk and two brands of yogurt (from two different manufacturers) were selected. Each sample analysis was done in triplicate. Statistical analysis was conducted using Sigma Stat 1.0 (Jandel Corp., San Rafael, Calif.). Appropriate comparisons were made using the Student–Newman–Keuls test for multiple comparisons. Comparisons were made only within the same product over storage time.

## RESULTS AND DISCUSSION

Viability of bifidobacteria and lactic acid bacteria were monitored in three separate batches obtained from different lots and different times of commercial A/B milk over an 18-day refrigerated storage period (Fig. 1). A significant decrease ( $P < 0.05$ ) in the bifidobacteria population was observed at 3 days prior to product expiration; however, the viable bifidobacteria population in commercial A/B milk remained above  $10^6$  CFU/ml until the product expired. Six days after the expiration date, bifidobacteria population dropped below  $10^6$  CFU/ml (Fig. 1A).

The changes in lactic acid bacterial population during the duration of the study were not statistically significant (Fig. 1B). The pH of the A/B milk remained at 6.6 or above during the study (data not shown). Sanders et al. (27) reported that populations of streptococci, lactobacilli, and bifidobacteria were very stable in fluid milk throughout 21 days of incubation. The pH of both inoculated and control (uninoculated) milks dropped similarly from pH 6.8 to 6.5, suggesting metabolic activity of psychrotrophic microorganisms. Reuter (22) reported on the fermented and culture-containing (unfermented) milk sold in Japan and concluded that these products had sufficient counts ( $>10^6$  CFU/ml) of bifidobacteria even after 15 days of storage. The unfermented milk containing cultures had sufficient counts up to 18 days. *B. bifidum* was slightly more stable than *B. breve* in the products they investigated. Lankaputhra et al. (14) observed that viability of *B. infantis* in 12% skim milk at pH 4.3 were decreased by 30% after 12 days of storage at 4°C. After 24 days at the same temperature the counts decreased by more than 82%. Medina and Jordano (16) reported on the bifidobacterial counts of fermented milk produced in Spain stored at 7°C. They observed a 93% de-

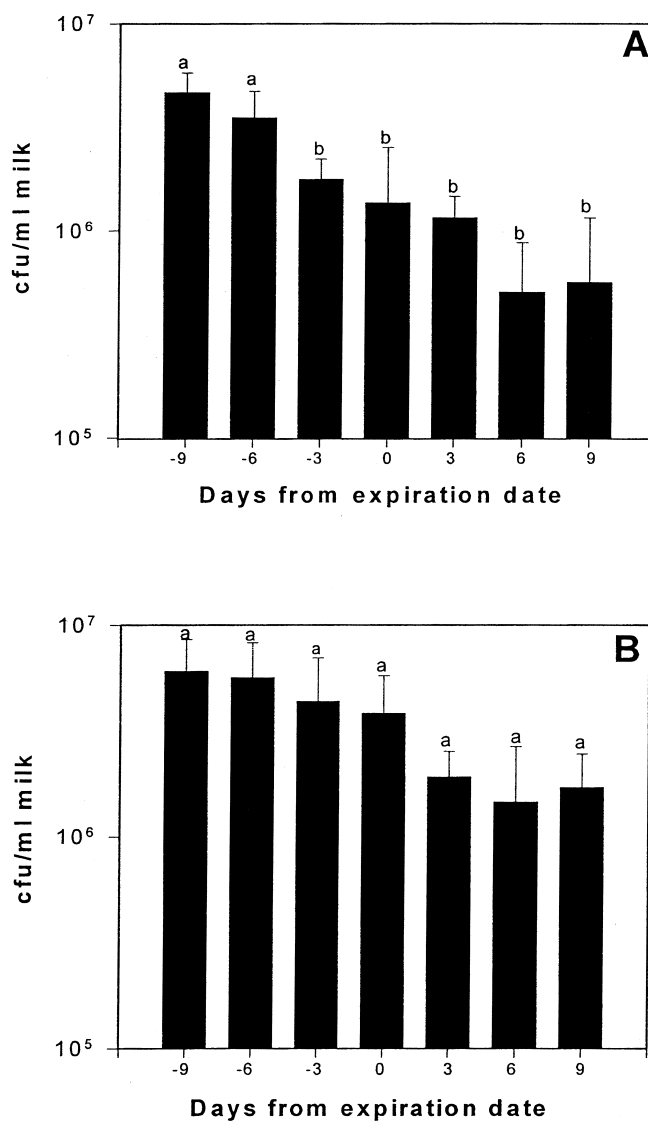


FIGURE 1. Viability of bifidobacteria (A) and lactic acid bacteria (B) in commercial A/B milk during 18 days of refrigerated storage at 4°C. 0 = expiration day; -9, -6, -3 = days prior to product expiration; 3, 6, 9 = days past expiration. Bars with different letters are significantly different ( $P \leq 0.05$ ),  $n = 3$  for all data points (three separate batches of milk purchased at different times), and each sample was plated in triplicate.

crease in the bifidobacterial population when the product expired. In our study we observed a 71% reduction in bifidobacterial population at the time of product expiration. The A/B milk in our study was not a fermented milk as in Medina and Jordano's (16) study. Thus, acid injury to the organism was avoided. The lactic acid bacteria population in the A/B milk decreased by 37% at expiration time; however, these counts were above  $10^6$  CFU/ml at the time of expiration.

Viability of bifidobacteria and lactic acid bacteria in commercial brand A and B yogurts were assessed over a 6-week period (Figs. 2 and 3). In brand A yogurt, although a significant decrease ( $P < 0.05$ ) was observed 1 week past the product expiration day, viable population of bifidobacteria remained above  $10^6$  CFU/g until 2 weeks past the product expiration (Fig. 2A). It was only 3 weeks after the

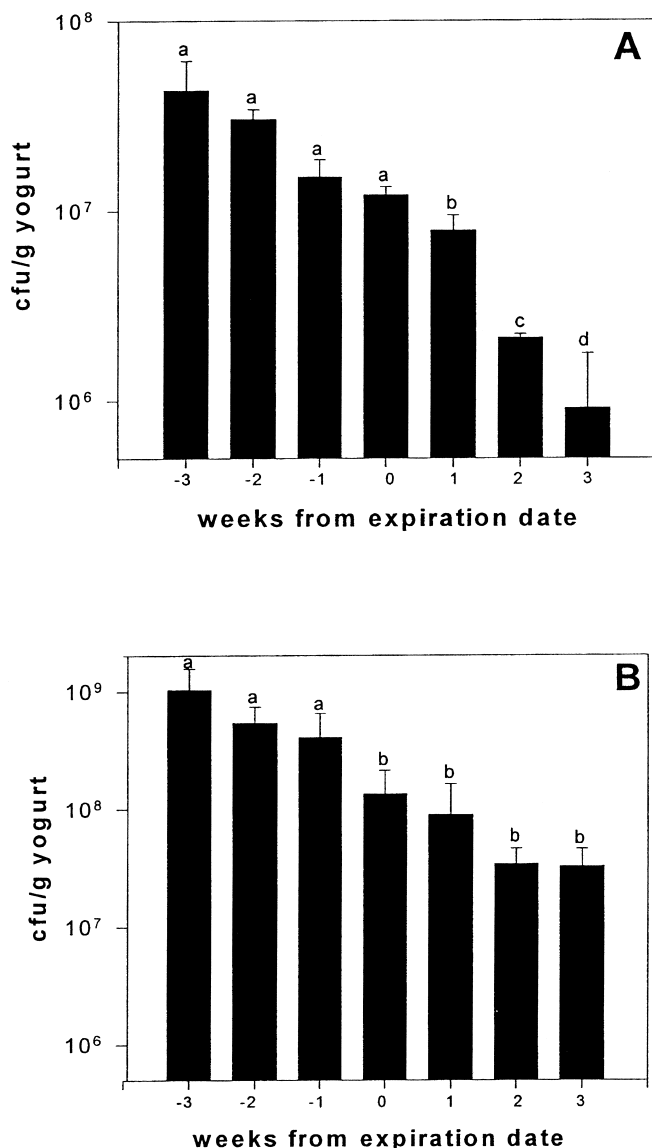


FIGURE 2. Viability of bifidobacteria (A) and lactic acid bacteria (B) in commercial brand A yogurt during 6 weeks of refrigerated storage at 4°C. 0 = expiration day; -6, -3, -1 = weeks prior to product expiration; 1, 3, 6 = weeks past expiration. Bars with different letters are significantly different ( $P \leq 0.05$ ),  $n = 3$  for all data points (three separate batches of yogurt purchased at different times), and each sample was plated in triplicate.

product expired that the population dropped below  $10^6$  CFU/g. The lactic acid bacterial population was maintained above  $10^7$  CFU/g during the duration of the study, although a significant decline ( $P < 0.05$ ) was observed on the expiration day of the product (Fig. 2B). Bifidobacteria and lactic acid bacteria populations had decreased 64% and 87%, respectively, when the product expired. The pH of the yogurt was 4.23 in the initial 5 weeks but dropped ( $P < 0.05$ ) to 4.18 the last 2 weeks of the study.

Viable bifidobacterial population in commercial brand B yogurt steadily declined during refrigerated storage (Fig. 3A). This decline was significant ( $P < 0.05$ ) at 1 week prior to product expiration and again at the date of expiration; however, the bifidobacterial population remained above  $10^6$  CFU/ml until 2 weeks past the expiration. The lactic acid

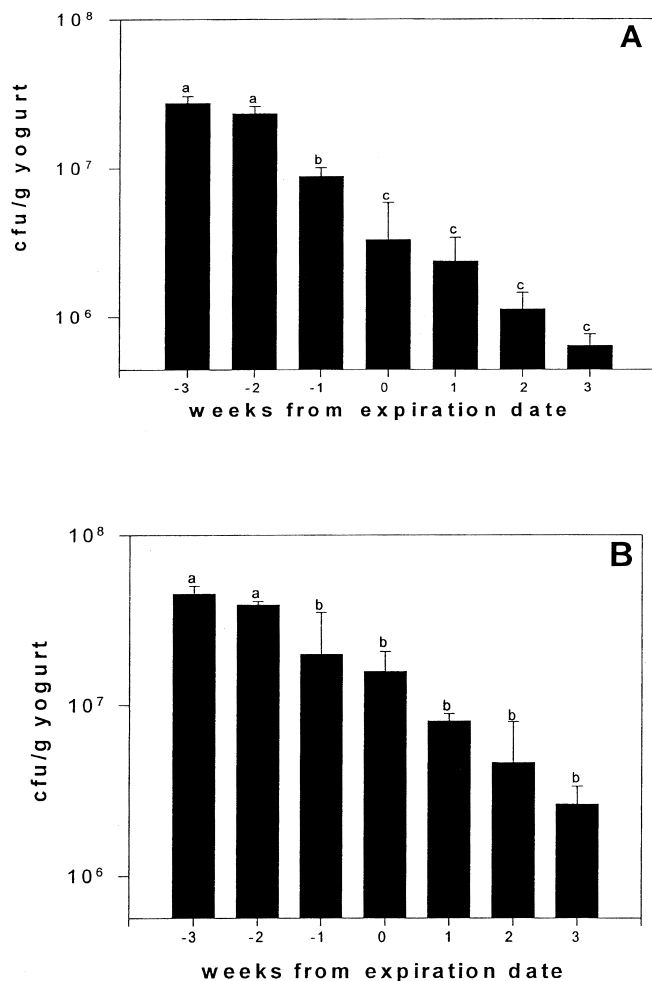


FIGURE 3. Viability of bifidobacteria (A) and lactic acid bacteria (B) in commercial brand B yogurt during 6 weeks of refrigerated storage at 4°C. 0 = expiration day; -6, -3, -1 = weeks prior to product expiration; 1, 3, 6 = weeks past expiration. Bars with different letters are significantly different ( $P \leq 0.05$ ),  $n = 3$  for all data points (three separate batches of yogurt purchased at different times), and each sample was plated in triplicate.

bacteria population in brand B yogurt declined significantly ( $P < 0.05$ ) 1 week prior to product expiration date (Fig. 3B); however, the counts were again above  $10^6$  CFU/g during the duration of the study. Bifidobacterial and lactic acid bacterial populations decreased 88% and 65%, respectively, on the product expiration day. The pH for this brand of yogurt initially was 4.20 and dropped ( $P < 0.05$ ) to 4.17 at 1 week past the expiration date. It appeared that brand B yogurt had a lower lactic acid bacteria population than brand A. This difference may be due to differences in the amounts of initial inoculum, product processing conditions, differences in lactic acid bacteria strains, as well as differences in the pH values of the two products.

Bifidobacteria and lactic acid bacteria counts reported herein are consistent with our previous report on viability of these organisms in yogurt manufactured in the laboratory from reconstituted nonfat dry milk using commercial yogurt starter cultures for animal feeding studies (8, 31). Shah et al. (28) reported on initial bifidobacterial counts in five brands of commercial yogurt purchased in Australia

lia. In two of the five brands of yogurts purchased, counts were  $10^6$  to  $10^7$  CFU/g, but in the remaining three, they were  $<10^3$  CFU/g, indicating significant variability in counts in similar products. All the products showed a constant decline in numbers of bifidobacteria after production. At the end of 5 weeks of refrigerated storage, there were very few viable cells of bifidobacteria in any of the products.

Dave and Shah (4) reported on viability of probiotic bacteria in yogurts made from commercial starter cultures. They observed a three-log reduction in bifidobacteria counts from an initial of  $10^6$ /g or greater during the 35 days of refrigerated storage. The bifidobacterial population was higher in yogurt stored in glass bottles than in plastic cups; this difference in viability was attributed to the differences in oxygen permeability of the two packaging materials. Dave and Shah (6) reported that viability of bifidobacteria can be increased by three logs during 35 days of refrigerated storage by incorporation of whey protein concentrate, acid casein hydrolysate, or tryptone. Highest viability was observed in yogurts supplemented with whey protein concentrate. Micanel et al. (18) also reported on the viability of bifidobacteria in Australian yogurts sold commercially. Of the three products investigated, one maintained high levels ( $>10^6$  CFU/g), another declined from  $1.5 \times 10^5$  to  $<10^3$  CFU/g within 2 weeks after manufacture, and no viable bifidobacteria ( $<10^3$  CFU/g) were detected in the third product. Reuter (22) observed adequately high bifidobacteria counts ( $>10^6$  CFU/g) in commercial yogurts manufactured and sold in Germany and France even in yogurts with a pH as low as 4.0, suggesting better anaerobic conditions during processing and selection of more acid-tolerant strains by the industry in these countries. These numbers of organisms were consistent with the counts presented herein. Although bifidobacterial levels were variable in products investigated, they were always above  $10^6$  CFU/ml or g at the time of expiration, which is the recommended dose (13) to receive the health benefits of these organisms.

In summary, commercial fluid milk and yogurts manufactured in the U.S. tested here contained bifidobacterial populations at the recommended concentrations to receive the potential health benefits of these organisms. Differences in strains and production procedures among manufacturers might contribute to the slight differences in viability and activity of bifidobacteria as well as the lactic cultures in dairy products. While the study conducted here is limited in scope, it suggests that the dairy industry has been successful in identifying appropriate strains and handling to achieve prolonged viability and high numbers of bifidobacteria in commercial products. Further research is needed to clarify the potential health benefits received by ingesting viable bifidobacteria in adequate numbers with appropriate metabolic activities.

#### ACKNOWLEDGMENTS

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