SURVIVAL OF LATE EXPONENTIAL PHASE AND STATIONARY PHASE CELLS OF *LACTOBACILLUS ACIDOPHILUS* AT 7°C

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Story in Brief

The viability and ß-galactosidase activity of three strains of *Lactobacillus acidophilus* were monitored over a 28 day storage period at -196°C and during subsequent storage in milk at 7°C. Cells were grown in broth and harvested after 16 to 18 hours and after 22 to 24 hours. No decrease in viability or ß-galactosidase activity was observed for any strain during storage at -196°C regardless of harvest time. The cells from *L. acidophilus* 223 harvested after 24 hours decreased less during storage at 7°C than did the cells harvested after 18 hours. There were little or no differences in the decline in viability between the two harvest times for the other two strains. All strains decreased in ß-galactosidase activity during storage in milk at 7°C with no difference between the two harvest times.

(Key Words: *Lactobacillus acidophilus*, Viability, ß-galactosidase Activity.)

Introduction

Several potential health benefits have been associated with the consumption of acidophilus milk products. One of these potential benefits is improved digestion of lactose in persons who are lactose maldigestors. The bacteria may provide the enzyme ß-galactosidase which is responsible for hydrolyzing lactose in the intestinal tract therefore enabling lactose maldigestors to digest it. Other potential health benefits include control of intestinal infections, control of serum cholesterol levels, and control of certain types of intestinal cancer.

A readily-available source of *L. acidophilus* is nonfermented acidophilus milk. In order for this product to provide potential health benefits to consumers, the organism must remain viable and biologically active during preparation and storage of the milk. The growth conditions used for producing cells of lactobacilli can influence the organism's ability to survive both frozen and subsequent refrigerated storage. The objective of this study was to determine the influence of harvest time on the viability and ß-galactosidase activity of *L. acidophilus* during frozen storage and subsequent refrigerated storage.

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Materials and Methods

The cells of *L. acidophilus* were grown in peptonized milk nutrient (PMN) broth maintained at 37°C and at pH 5. The broth was inoculated at 1% using a freshly prepared culture of the desired strain of *L. acidophilus*. One liter samples were removed aseptically at sample times selected to coincide with the late log phase of growth (16 to 18 hours) and six hours into the stationary phase of growth (22 to 24 hours) for each culture. The appropriate sample times for each culture were determined in preliminary experiments in which populations were determined hourly.

Cells were harvested from the 1-liter samples by centrifugation and resuspended in twice their weight of cold, sterile, reconstituted 10% nonfat dry milk. The resulting concentrated cultures were distributed into sterile cryogenic vials. All vials but one were submerged in liquid nitrogen for storage at -196°C. The remaining vial was held in an ice water solution and assayed within 2 hours.

On day 28, one vial was removed from the liquid nitrogen for analysis and another was removed to prepare the nonfermented acidophilus milk containing approximately 2×10^7 *L. acidophilus*/ml. The nonfermented milk was stored at 7°C as assayed on days 0, 7, 14, 21, and 28.

The assays included measurement of total numbers and ß-galactosidase activity. Total numbers of lactobacilli were determined using PMN agar. ß-galactosidase activity was determined using o-nitrophenyl ß-D-galactopyranoside (ONPG) as a substrate by procedures routinely used in our lab. Three strains (107, 223, 606) of *L. acidophilus* were included. Experiments for each strain were replicated three times.

Analysis of variance for each set of data was conducted as a split plot in a randomized block design to determine whether significant differences existed. Each fermentation was a block, harvest time was the main unit treatment, and days of storage was the subunit treatment. Least significant difference analyses were used to compare means for significant differences at the 5% level of confidence.

Results and Discussion

During the 28 day storage period in liquid nitrogen, there were no declines ($P>.20$) in total numbers or ß-galactosidase activity in any of the three strains at either harvest time.

ß-galactosidase activity and total numbers declined during storage in milk at 7°C. There was a difference ($P<.05$) in the initial ß-galactosidase activity between the two harvest times for *L. acidophilus* 107, but there were no other differences ($P>.05$) between the two harvest times for any of the three strains at any day of storage (Table 1). The ß-galactosidase activity of all three strains decreased with increased storage time. The ß-galactosidase activity of cells of strain 107 harvested in the late log phase (16 hr) declined ($P<.05$) after 14 days of storage while the cells harvested in the stationary phase (22 hr) declined

Table 1. ß-galactosidase activity of three strains of *Lactobacillus acidophilus* in nonfermented acidophilus milk stored at 7 C.

Days at 7 C	µmoles ONP released/min/ml[a]					
	L. acid 107		*L. acid* 606		*L. acid* 223	
	16 hr[b]	22 hr[b]	18 hr	24 hr	18 hr	24 hr
0	.16^{d}	.27^{c}	.12^{c}	.10^{cde}	.09^{cd}	.09^{cd}
7	.14de	.13^{def}	.10^{cde}	.08^{fg}	.08^{cde}	.10^{c}
14	.12^{efg}	.13^{def}	.11^{ed}	.09^{def}	.08^{cde}	.07^{def}
21	.08^{h}	.10^{fgh}	.10^{def}	.08^{ef}	.06^{ef}	.05^{f}
28	.09^{gh}	.10^{fgh}	.07^{f}	.07^{f}	.05^{f}	.05^{f}
SE days[i]	.018		.030		.009	
SE hours[i]	.017		.010		.011	

[a] Each value represents the mean of three trials.

[b] Time of harvest of cells for preparation of concentrated culture; 16 to 18 hr = late log phase and 22 to 24 hr = stationary phase.

c,d,e,f,g,h Numbers with different superscripts within 1 strain are different (P<.05).

[i] SE days = standard error for days; SE hours = standard error for harvest time.

Table 2. Total numbers of three strains of *Lactobacillus acidophilus* in nonfermented acidophilus milk stored at 7 C.

Days at 7 C	Log_{10} cfu/ml[a]					
	L. acid 107		*L. acid* 606		*L. acid* 223	
	16 hr[b]	22 hr[b]	18 hr	24 hr	18 hr	24 hr
0	7.76[c]	7.44[c]	7.99[c]	7.88[c]	7.61[c]	7.76[c]
7	6.06[de]	6.44[d]	7.73[cd]	7.64[cd]	7.53[c]	7.71[c]
14	5.91[de]	6.25[d]	7.74[cd]	7.74[cd]	7.50[c]	7.77[c]
21	5.56[e]	5.49[ef]	7.74[cd]	7.76[cd]	7.31[c]	7.47[c]
28	4.91[fg]	4.75[g]	7.54[d]	7.48[d]	7.48[c]	7.58[c]
SE days[i]	.298		.192		.171	
SE hours[i]	.301		.172		.226	

[a] Counts on PMN agar; each value represents the mean of three trials.

[b] Time of harvest of cells for preparation of concentrated culture; 16 to 18 hr = late log phase and 22 to 24 hr = stationary phase.

[c,d,e,f,g,h] Numbers with different superscripts within 1 strain are different ($P<.05$).

[i] SE days = standard error for days; SE hours = standard error for harvest time.

after 7 days of storage. Numbers of viable cells of strain 606 declined (P<.05) after 28 days of storage for the cells harvested from the late log phase (18 hr) and the stationary phase (24 hr). The ß-galactosidase activity for cells from both harvest times for *L. acidophilus* 223 declined (P<.05) after 21 days of storage at 7°C.

There were no differences (P>.05) in total numbers between the two harvest times during the 28 day storage period at 7°C for any of the three strains (Table 2). Total numbers of *L. acidophilus* 107 declined (P<.05) after 7 days of storage at 7°C for both harvest times. Total numbers of *L. acidophilus* 606 declined (P<.05) after 28 days of storage for both harvest times while total numbers of *L. acidophilus* 223 from both harvest times did not decline (P>.05) over the entire 28 day storage period.

Because there were no significant declines in total numbers of *L. acidophilus* 223 during the 28 day storage period at 7°C, the storage period was extended for an additional 35 days to observe how long the cells would remain viable (Figure 1). The decline of total numbers of cells harvested in the stationary phase (24 hr) of growth was not (P<.05) until day 56 while that for the cells harvested in the late log phase (18 hr) occurred much sooner.

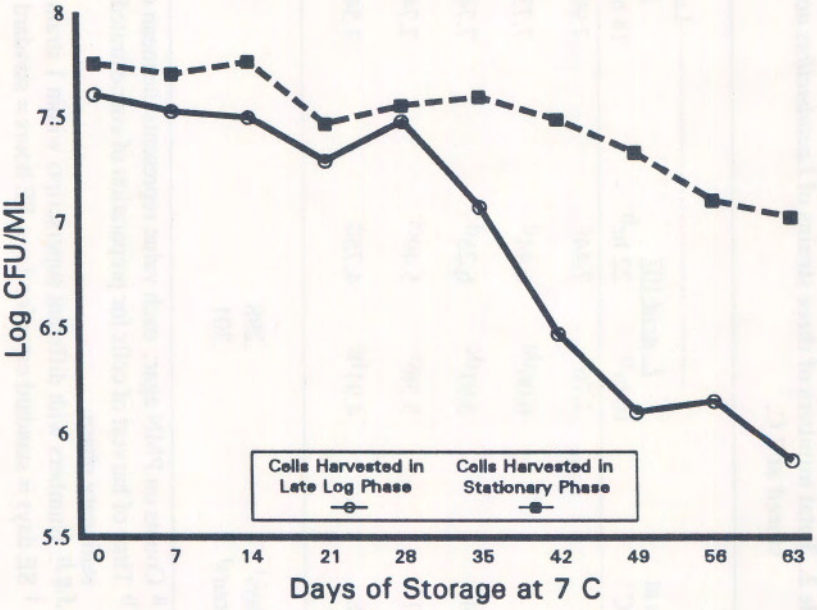

Figure 1. Total numbers of *Lactobacillus acidophilus* 223 in nonfermented acidophilus milk at 7 C.

However, it would not be likely that nonfermented acidophilus milk meant for human consumption would be stored for this amount of time.

These data indicate that harvesting cells during the stationary phase of growth had no beneficial or detrimental effect on the viability or ß-galactosidase activity of the cells during storage at -196°C or during subsequent storage in milk at 7°C. There could be a benefit to harvesting cells in the stationary phase of growth if they were going to be used in a product that would be subjected to longer storage times. This and other research in our lab has indicated that it is possible to supply consumers with viable *L. acidophilus* in the form of nonfermented acidophilus milk.