INFLUENCE OF GROWTH AT DIFFERENT pH LEVELS ON STORAGE STABILITY OF *LACTOBACILLUS ACIDOPHILUS* IN REFRIGERATED MILK

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Story in Brief

A broth medium was used to grow *Lactobacillus acidophilus* at pH 5.0, 5.5, 6.0, 6.5 or 7.0. Concentrated cultures prepared when the culture had reached maximum population in the fermentor were stored at -196 C for 28 days with no loss in viability or bile resistance.

After 28 days of storage the frozen concentrated cultures were thawed and added to sterile, cold 10 percent non-fat milk solids at a population of approximately 2 x 10^6/ml and stored at 5 C. Cells from the culture which had been grown at pH 5 were much more stable during storage than were cells from cultures grown at the higher pH levels.

(Key Words: *Lactobacillus acidophilus*, Concentrated cultures, Acidophilus-milk.)

Introduction

Frozen concentrated cultures used for the preparation of a non-fermented food, such as acidophilus milk, should possess a number of desirable attributes in order to serve as a beneficial dietary adjunct. They should maintain stability during growth, remain viable and bile resistant during freezing and storage as a concentrated culture and during subsequent storage in the food product. Growth conditions including medium composition, pH temperature, oxidation - reduction potential and incubation time can influence such desirable attributes of the culture. Additionally, freezing and storage conditions also can influence the desirable attributes of the culture.

The purpose of this study was to determine the effect of pH during growth on the survival of cells of *Lactobacillus acidophilus* frozen and stored at -196 C and subsequently during storage in refrigerated pasteurized low-fat milk.

Materials and Methods

Cells of *Lactobacillus acidophilus* NCFM were grown at 37 C in a fermentor containing 4 liters of PMN broth with pH being maintained by an automatic pH control unit adding a sterile neutralizer solution as needed. The neutralizer solution contained 20 percent Na_2CO_3 in 10 percent NH_4OH. The PMN broth contained 5 percent Peptonized Milk Nutrient (Sheffield Products), 2 percent Primatone (Sheffield Products), 2 percent lactose, 0.1 percent yeast extract and 0.1% Tween 80 (polysorbitan monoleate). The initial pH of the medium was 6.0 and was adjusted to the desired level prior to inoculation by adding either sterile 20 percent lactic acid solution or the alkaline neutralizer solution as required. The four liters of broth was

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inoculated with 40 ml of freshly prepared culture of L. acidophilus NCFM which had been incubated at 37 C for 18 hr. The inoculated medium was incubated at 37 C with the pH being maintained at the desired level throughout the incubation period. Starting at 10 hr, samples were taken from the fermentor at 2 hr intervals so the growth could be monitored by turbidimetric readings and by plate counts. Five pH levels were evaluated: pH 5.0, 5.5, 6.0, 6.5 and 7.0. Three replicates of each level were performed.

One liter of the broth culture was removed from the fermentor when turbidimetric readings indicated maximum growth had been reached. Cells were harvested by centrifugation and a concentrated culture was prepared by resuspending the cell pellet in twice its weight of sterile cold 10 percent non-fat milk solids. The concentrated culture was dispensed in 2 g quantities, frozen and stored in liquid nitrogen. Plate counts on PMN agar were made prior to freezing and after 28 days storage. PMN agar was prepared by adding 15 g of agar to one liter of PMN broth prior to sterilization. The plates were incubated aerobically at 37 C for 48 hr.

On the 28th day of frozen storage, concentrated culture was removed and thawed by immersion in tap water at 25 C. Sufficient concentrated culture was then added to the cold milk to attain a population of approximately 2 x 10^6/ml. The bottles were stored at 5 C and removed on days 1, 7, 14 and 21 to determine numbers of lactobacilli by plate counts on PMN agar.

Results and Discussion

The highest numbers of cells and greatest stability during growth for 24 hr was observed for cells grown at pH 5.0, while the lowest numbers and greatest fluctuation during growth was at pH 7.0 (Figure 1). Growth at pH 5.5, 6.0 and 6.5 produced higher populations than growth at pH 7.0 but appeared to fluctuate or decline more than did cultures maintained at pH 5.0. The culture grown at pH 5 had attained maximum population at 10 hr.

No significant changes ($P<.05$) were observed in total numbers of lactobacilli or bile resistant lactobacilli during frozen storage (Table 1). However, concentrated cultures produced from cells grown at pH 7.0 exhibited a slight decline in number during the 28 days of storage at -196 C.

Table 1. Survival of Lactobacillus acidophilus NCFM in concentrated cultures during storage at -196 C.

	Log_{10} Count/g[a]				
Days	pH 5.0[b]	pH 5.5	pH 6.0	pH 6.5	pH 7.0
0	10.50	10.57	10.47	10.31	10.12
1	10.53	10.56	10.41	10.31	9.96
28	10.48	10.58	10.42	10.28	10.04

[a]Each value = average from 3 trials.
[b]pH at which cells were grown.

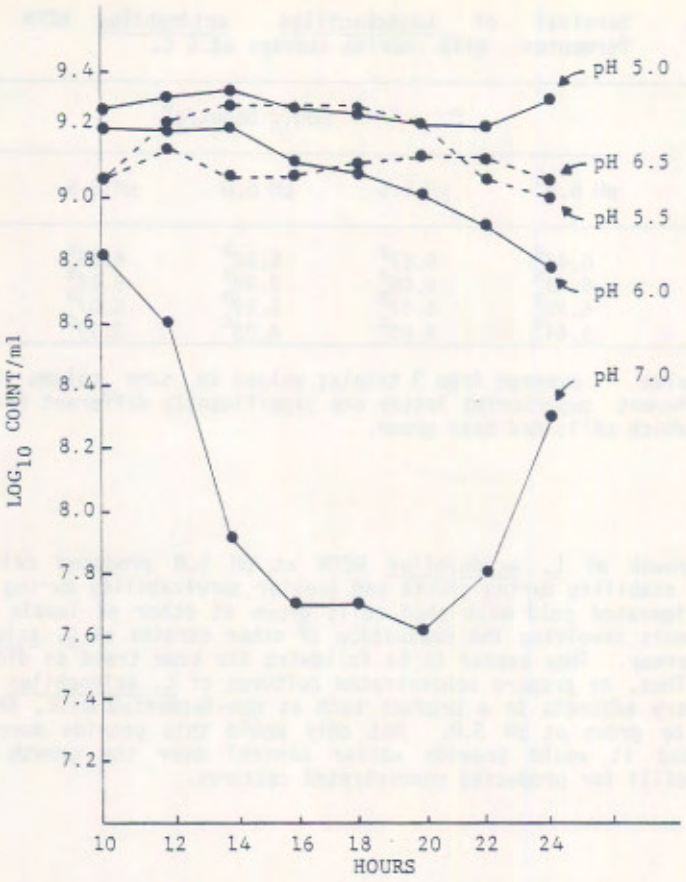

Figure 1. Comparison of growth of Lactobacillus acidophilus NCFM at various pH levels in PMN broth (Each point represents an average from 3 trials).

Survivability of cells in refrigerated milk was determined by enumeration of lactobacilli at 7 day intervals for 21 days after inoculation with the concentrated culture. Results show considerable differences in survival of cells grown at different pH levels. Cells at pH 5.5, 6.0, 6.5 and 7.0 showed steady decline in numbers throughout the period tested (Table 2). The decreases were significant for days 14 and 21 ($P<.05$).

Cells grown at pH 5.0 showed little or no decrease in cell number after 14 days storage in refrigerated milk at 5 C. Evidence of decreased stability ($P<.05$) was observed for cells stored 21 days but decreases were not as great as at other pH levels.

Table 2. Survival of Lactobacillus acidophilus NCFM in non-fermented milk during storage at 5 C.

Days	Log_{10} Count/g[a]				
at 5 C	pH 5.0[b]	pH 5.5	pH 6.0	pH 6.5	pH 7.0
1	6.47[a]	6.37[a]	6.58[a]	6.26[a]	6.30[a]
7	6.43[a]	6.06[a]	6.39[a]	6.14[a]	6.05[ab]
14	6.30[a]	5.37[b]	5.59[b]	5.07[b]	5.38[b]
21	5.44[b]	4.05[c]	4.09[c]	3.99[c]	4.96[c]

[a]Each value = average from 3 trials; values in same column followed by different superscript letter are significantly different ($P<.05$).
[b]pH at which cells had been grown.

Growth of L. acidophilus NCFM at pH 5.0 produced cells with greater stability during growth and greater survivability during storage in refrigerated cold milk than cells grown at other pH levels tested. Experiments involving the evaluation of other strains of L. acidophilus are underway. They appear to be following the same trend as did strain NCFM. Thus, to prepare concentrated cultures of L. acidophilus for use as dietary adjuncts in a product such as non-fermented milk, the cells should be grown at pH 5.0. Not only would this provide more stable cells but it would provide easier control over the growth of the lactobacilli for producing concentrated cultures.