

Improvement of Lactose Digestion by Humans Following Ingestion of Unfermented *Acidophilus* Milk: Influence of Bile Sensitivity, Lactose Transport, and Acid Tolerance of *Lactobacillus acidophilus*

AZLIN MUSTAPHA,* TIANAN JIANG,[†] and DENNIS A. SAVAIANO^{‡,1}

*Department of Food Science and Human Nutrition,
University of Missouri, Columbia 65211

[†]Department of Pediatrics,
University of Iowa, Iowa City 52242

[‡]School of Consumer and Family Sciences,
Purdue University, West Lafayette, IN 47907

ABSTRACT

The influence of bile sensitivity, lactose transport, and acid tolerance of *Lactobacillus acidophilus* on in vivo digestion of lactose was investigated. Four strains of *L. acidophilus* exhibiting varied degrees of lactose transport, β -galactosidase activity, and bile sensitivity were used to prepare unfermented acidophilus milks. Lactose malabsorption was evaluated by measuring breath H₂ excretion of 11 lactose maldigesting subjects following ingestion of four acidophilus test milks. Test meals were fed in a randomized double-blind protocol. Consumption of acidophilus milk (2% fat) containing strains B, N1, and E significantly reduced mean total H₂ production compared with that of the control reduced-fat (2% fat) milk, but milk containing strain ATCC 4356 did not differ from the control. Acidophilus milk containing *L. acidophilus* N1 was the most effective of the four acidophilus milks in improving lactose digestion and tolerance. Strain N1 exhibited the lowest β -galactosidase activity and lactose transport but the greatest bile and acid tolerance of the four strains. The results indicated that bile and acid tolerance may be important factors to consider when *L. acidophilus* strains are selected for improving lactose digestion and tolerance.

(**Key words:** lactose digestion, *Lactobacillus acidophilus*, unfermented acidophilus milk)

Abbreviation key: A = absorbance (used with subscript indicating wavelength in nanometers), β -GAL = β -galactosidase, **4356 milk** = unfermented acidophilus milk made with *Lactobacillus acidophilus* ATCC

4356, **B milk** = unfermented acidophilus milk made with *L. acidophilus* B, **E milk** = unfermented acidophilus milk made with *L. acidophilus* E, **N1 milk** = unfermented acidophilus milk made with *L. acidophilus* N1.

INTRODUCTION

Consumption of cultured dairy products by lactose-intolerant individuals is known to alleviate or decrease the symptoms of intolerance that are associated with the consumption of milk. This effect is attributed to the presence of intracellular bacterial β -galactosidase (β -GAL), which can survive the passage through the gastrointestinal tract and hydrolyze lactose in vivo. Numerous reports have established the improvement of lactose digestion by lactose maldigesters consuming yogurt and fermented milks (10, 17, 25). The benefits of unfermented acidophilus milk made with viable *Lactobacillus acidophilus* cells, however, have been controversial (9, 13, 18, 20, 23, 25). Previously unsuccessful attempts at formulating effective acidophilus milks may be attributed to low or undetectable concentrations of β -GAL resulting from the use of frozen concentrated starter culture, inappropriate substrates for culture growth, insufficient cell concentrations, or extended storage of the products (20, 23, 25). In addition to proper preparation of acidophilus milk, the selection of the strain of *L. acidophilus* used in studies on lactose maldigestion may be critical because intrinsic cellular properties, such as β -GAL activity, lactose transport, and bile and acid tolerance, vary among different strains.

The presence of β -GAL is necessary, but is not the limiting factor involved in promoting in vivo lactose digestion (13, 17). For most lactobacilli, lactose metabolism is governed by the presence of a lactose permease, which transports lactose into the cell, and β -GAL, which hydrolyzes lactose into glucose and

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¹Send correspondence to D. A. Savaiano, Purdue University, School of Consumer and Family Sciences, 1260 Stone Hall Room 110, West Lafayette, IN 47907-1260.

galactose. Thus, a possible limiting factor in effective in vivo digestion of lactose is the cellular rate of lactose uptake. Theoretically, the rate of lactose hydrolysis is directly related to the rate of lactose transport by the cell. However, rapid rates of lactose transport could also saturate the level of intracellular lactose, resulting in limited hydrolysis (17).

Another limiting factor may be the sensitivity or tolerance of the cultures to bile. Bile tolerance of *L. acidophilus*, whereby cells are not lysed by bile, has been suggested as being important for determining the survival and colonization of this organism in the colon (6). Bile salts have been shown to enhance the lactose-hydrolyzing ability of this organism (3, 21) by rendering the cell membrane more permeable, thus enhancing the accessibility of lactose to the intracellular β -GAL. However, McDonough et al. (18) found that unfermented acidophilus milk made with sonicated *L. acidophilus* cells resulted in higher concentrations of β -GAL and a correspondingly greater improvement of lactose digestion than occurred with unfermented acidophilus milk made with intact cells. Lin et al. (13) demonstrated a modest improvement in lactose digestion from milk containing bile-sensitive strains of *L. acidophilus* cells compared with results using more bile-tolerant strains. These findings may indicate that bile sensitivity, or bile-induced cell lysis, may be a more important factor to consider when strains of *L. acidophilus* are being selected to aid in vivo digestion of lactose.

Except for β -GAL activity, factors determining the ability of *L. acidophilus* cells to aid in vivo digestion of lactose have not been sufficiently studied. To select strains that maximize lactose digestion from unfermented acidophilus milk, it is necessary to understand the factor or factors that influence in vivo digestion of lactose. This study was conducted to determine whether lactose transport or bile sensitivity of *L. acidophilus* interact with β -GAL activity to improve in vivo digestion of lactose. If either or both of these attributes are limiting, a rationale could be developed for the selection of *L. acidophilus* strains to ensure a maximum rate of in vivo digestion of lactose from unfermented acidophilus milk.

MATERIALS AND METHODS

Bacterial Strains and Growth Conditions

All *L. acidophilus* strains, except strain LA-1, which was a kind gift from Chr. Hansen's Laboratory, Inc. (Milwaukee, WI), were from the University of Minnesota culture collection. Strain LA-1 was con-

firmed to be the same as strain NCFM (Chr. Hansen's Laboratory, 1996, personal communication). All strains were maintained by biweekly transfers in modified lactobacilli MRS broth (made from individual ingredients according to the formulation by Difco, Detroit, MI) containing 2% lactose as the sole carbohydrate source and an adjusted pH of 5.4. Cultures were grown at 37°C for 18 h and transferred twice prior to experiments.

Growth of *L. acidophilus* in Bile

Fresh 18-h cultures of *L. acidophilus* were inoculated at a level of 2% (vol/vol) into 10 ml of the MRS broth containing lactose and 0.3% bile (Oxgall; Sigma Chemical Co., St. Louis, MO). Growth of the cultures at 37°C was monitored for 8 h by recording the absorbance (A) at 625 nm (A_{625}) hourly (model DU-8 spectrophotometer; Beckman Instruments, Fullerton, CA). Bile sensitivity of the strains was recorded as the difference in A_{625} at 8 h between growth in the control medium (containing 0% bile) and growth in 0.3% bile. The data were means of duplicate batches of milk with duplicate absorbance measurements.

Preparation of Cell-Free Enzyme Extracts

Twenty milliliters of an 18-h culture were harvested and washed twice in 20 mM phosphate buffer (containing 5 mM $MgSO_4$), pH 7.0, at 12,000 $\times g$ and 4°C for 10 min each (model J2-21; Beckman Instruments, Fullerton, CA). Washed pellets were resuspended in 10 ml of the same buffer, and suspensions were kept on ice for not more than 15 min. Cells, placed in ice in a secondary beaker, were sonicated for four 1-min intervals (Sonifier; Branson Instruments, Danbury, CT). Sonicated cell suspensions were centrifuged to remove cell debris (12,000 $\times g$ at 4°C for 15 min) and filter-sterilized through 0.45- μm pore filter discs (Acrodisc; Gelman Sciences, Inc., Ann Arbor, MI) to obtain cell-free enzyme extracts. Extracts were stored in ice at 4°C overnight before assay for β -GAL.

Determination of β -GAL Activity

The β -GAL activity of pure cultures was determined according to the method of Lin et al. (14) using 5 mM *o*-nitro- β -D-galactopyranoside (Sigma Chemical Co.) as the substrate. The assay was performed using the Beckman DU-8 spectrophotometer programmed for kinetic determinations. Product formation was measured at 37°C until an increase in A_{420} was linear for at least 10 min. One unit of β -GAL activity was defined as the amount of enzyme needed

to cleave 1 μmol of *o*-nitro- β -D-galactopyranoside/min at 37°C. Results were expressed as units per milliliter for total β -GAL activity and units per milligram of protein for specific β -GAL activity. Protein concentrations were determined by the Lowry method (15) using a protein determination kit (Sigma Chemical Co.). Total β -GAL activity of the acidophilus milks was determined in sonicated (four 1-min intervals in ice) test milks, and the results were expressed as units per milliliter of milk.

Determination of Lactose Uptake

Lactose uptake was assayed by a modified version of the method of Hutkins and Ponne (8). Intracellular and extracellular cell volumes were determined by the method of Nannen and Hutkins (19). Cells were grown to midlogarithmic phase, harvested, and washed twice in 50 mM phosphate buffer, pH 6.8, containing 10 mM MgCl and 0.5 mM dithiothreitol (12,000 $\times g$ at 4°C for 15 min). Cell pellets were resuspended in the same buffer to a final A_{625} of about 1.0 and iced until assayed. The assay was started by the addition of 0.0045 mCi/ml of [^{14}C]lactose (Amersham Corp., Arlington Heights, IL) to cell suspensions to attain a final concentration of 3 mM lactose (0.00014 mCi/ml). Reaction mixtures were incubated at 37°C in a shaking water bath for the duration of the assay (14 min). At five intervals, 1-ml samples were removed and centrifuged in 0.5 ml of silicone oil mixture (35% 556 fluid and 65% 550 fluid; Dow Corning, Midland, MI) for 1 min at 8000 $\times g$ and room temperature. Radioactivity of pellets and supernatants were counted in a Beckman model LS 5000 TD scintillation counter (Beckman Instruments, Fullerton, CA). Lactose uptake was expressed as millimolar concentrations of intracellular lactose over time (Figure 1).

Preparation of Acidophilus Milks

Four strains of *L. acidophilus* (ATCC 4356, B, N1, and E) were selected that had varying β -GAL activity, bile sensitivity, and lactose transport rate. Cells were grown for 18 h and then harvested by centrifugation at 12,000 $\times g$ and 4°C for 10 min. Cells were washed twice in sterile normal saline and recovered by centrifugation (12,000 $\times g$ and 4°C for 10 min). Washed cells were resuspended in a volume of reduced-fat milk (Kemps, Minneapolis, MN) equal to 1/100 of the original culture volume. Unfermented acidophilus milks were made by addition of this cell concentrate to 2% reduced-fat milk (Kemps) to attain cell numbers of 8 $\times 10^8$ /ml to 10 $\times 10^8$ /ml. Control

milk was uninoculated 2% reduced-fat milk (Kemps). Lactose contents of each milk were determined using a spectrophotometric assay (Boehringer-Mannheim Biochemicals, Indianapolis, IN). Test meals (400 ml) were fed within 24 h of preparation in a random, double-blind protocol to 11 lactose-maldigesting subjects. One test meal was fed per day, and a minimum of 3 d was allowed between treatments.

Determination of Acid Tolerance

Twenty milliliters of an 18-h culture (ATCC 4356, B, N1, and E) were harvested and washed twice with 0.1% peptone H₂O, pH 7.0, at 12,000 $\times g$ and 4°C for 10 min. Washed cell pellets were suspended in a volume of the same solution so as to increase cell numbers about 10-fold over that in the growth medium. The A_{625} of all four cell concentrates were adjusted to within 0.7 of one another. Cells were inoculated at a level of 2% (vol/vol) to each of 10 ml of 2% reduced-fat milk (Kemps), which had pH values of 1.0, 2.0, or 3.0. Concentrated hydrochloric acid was used to adjust the pH of the milk, and the milks were incubated at 37°C for up to 3 h. Control milk was unadjusted 2% milk (pH 6.7). At 0, 1, 2, and 3 h following inoculation, dilutions were made, and cells were pour plated in duplicate on lactobacilli MRS agar (Difco). Plates were incubated at 37°C under anaerobic conditions using BBL anaerobic jars (BBL Microbiological Systems, Division of Beckton Dickinson and Co., Cockeysville, MD) for 72 h before enumeration. The experiment was performed three separate times, and the means of data from all three trials were used to record cell numbers.

Subjects

Eleven healthy subjects (6 males and 5 females), aged 25 to 42, were identified as lactose maldigesters by a rise in breath H₂ concentration of >20 ppm following ingestion of 400 ml of milk (containing approximately 18 g of lactose). None of the subjects had suffered gastrointestinal illnesses or taken antibiotics 3 mo prior to or during the study. Each subject was instructed to fast at least 12 h prior to each test day. Subjects collected breath H₂ samples in 60-ml disposable plastic syringes and recorded hourly symptoms of intolerance (scale: 0 = no symptoms to 5 = severe symptoms) following ingestion of test meals. Diarrhea was monitored by recording the time and consistency of each bowel movement during the 24-h period following consumption of a test meal. Data were presented as the sum of ratings for h 1 to 8. Only water was consumed during the 8-h period.

Each subject served as his or her own control. All subjects provided written informed consent and completed the entire study. The study was approved by the Human Subjects Committee of the Institutional Review Board of the University of Minnesota.

H₂ and CO₂ Analysis

Lactose maldigestion was determined by analysis of end alveolar breath H₂ according to the method of Levitt and Donaldson (12). Hydrogen and CO₂ were measured by gas chromatography (model DP, Microlyzer Gas Analyzer, and model 24, AlveoLyzzer, respectively). Observed values for H₂ were corrected for atmospheric contamination of alveolar air by normalization of the observed CO₂ to 45 mm Hg, which is the venous partial pressure of CO₂ (10). To calculate changes in H₂ concentrations fasting H₂ concentrations were subtracted from subsequent hourly test values. Data were expressed as means (\pm SEM) for the 8-h period. Using a statistical software package (SYSTAT, Inc., Evanston, IL), the data were evaluated by ANOVA for a randomized block design in which a repeated measure protocol was performed. Fisher's least significant difference test was used to compare means.

RESULTS

Bile Sensitivity of β -GAL Activity of *L. acidophilus* Strains

Bile sensitivity of the *L. acidophilus* strains was defined as the difference in A₆₂₅ at 8 h between growth in 0% bile and growth in 0.3% bile (Table 1). Bile sensitivities ranged from 0.33 for strain Farr to 1.70 for strain LA-1, and total β -GAL activities

ranged from 0.5 U/ml for strain N1 to 1.45 U/ml for strain Farr. Bile sensitivity of strain 4356 (0.86) was the highest of the four strains evaluated in milk, although it did not differ ($P > 0.05$) from that of strain E (0.75). Strains B and E were also not different ($P > 0.05$) from one another in bile sensitivity. Total and specific β -GAL activities of cells grown in MRS-lac are also shown in Table 1. Specific activity values for β -GAL did not correspond to activity values for total β -GAL, as was previously reported for yogurt bacteria (14, 17). Both total and specific β -GAL activities of *L. acidophilus* strains indicated that strains 4356, N1, and LA-1 were not significantly different from one another ($P > 0.05$) and that strains B and E, and strains B and Farr, respectively, were also not significantly different from one another ($P > 0.05$). Because total β -GAL activity represented the level of the enzyme present in the test milks when consumed, all subsequent comparisons of strains used in test milks in this study refer to their total β -GAL activity instead of their specific β -GAL activity.

Lactose Transport of *L. acidophilus* Strains

Lactose transport of *L. acidophilus* strains is as shown in Figure 1. Although expressed as intracellular lactose in Figure 1, the lactose uptake data actually represented the total intracellular radioactive compounds, which included lactose as well as its metabolic products. Strain ATCC 4356 accumulated a total of 70 mM intracellular lactose at the end of 14 min. Strain B and Farr accumulated 50 and 40 mM lactose in 14 min, respectively. Strains E, LA-1, and N1 had similar concentrations of intracellular lactose, ranging from 14 to 19 mM, in 14 min.

TABLE 1. β -Galactosidase (β -GAL) activity and bile sensitivity of *Lactobacillus acidophilus* strains.¹

Strain	Total β -GAL activity ²		Specific β -GAL activity ²		Bile sensitivity ³	
	— (U/ml) —		— (U/mg) —		\bar{X}	SD
	\bar{X}	SD	\bar{X}	SD		
4356	0.63 ^c	0.17	2.94 ^a	0.28	0.86 ^b	0.07
B	1.23 ^{ab}	0.14	1.77 ^{bc}	0.16	0.67 ^c	0.03
N1	0.50 ^c	0.04	2.32 ^a	0.28	0.51 ^d	0.05
E	1.13 ^b	0.24	1.66 ^b	0.35	0.75 ^{bc}	0.02
Farr	1.45 ^a	0.18	1.91 ^c	0.19	0.33 ^e	0.02
LA-1	0.60 ^c	0.10	2.27 ^a	0.28	1.70 ^a	0.14

^{a,b,c,d,e}Means within a column without a common superscript letter differ ($P < 0.05$) by Fisher's least significant difference.

¹Duplicate trials.

²1 U = 1 μ mol of *o*-nitrophenyl released/min (n = 8).

³Difference in A₆₂₅ at 8 h between growth in 0 and 0.3% bile (n = 4).

Test Milks

Four strains were selected based on varying degrees of bile sensitivity, lactose transport, and β -GAL activities. These strains, ATCC 4356, B, N1, and E, were used to prepare unfermented acidophilus test milks (**4356 milk**, **B milk**, **N1 milk**, and **E milk**, respectively). Table 2 shows the mean bacterial counts, total β -GAL activities, and lactose contents of the test milks used in this study. Lactose contents ranged from 3.68 to 4.00% for the five test milks, and these values were not statistically significantly different from one another ($P > 0.05$). Thus, subjects consumed 15 to 16 g of lactose per test meal. The N1 milk had the lowest total β -GAL activity (0.5 U/ml), B and E milks had similar activities (0.8 U/ml), and 4356 milk had the highest activity (1.22 U/ml). The cell concentrations in the four acidophilus milks were similar and not significantly different from one another ($P > 0.05$). Bacterial growth and β -GAL activity were not detectable in the control milk.

Acid Tolerance of *L. acidophilus* Strains Used in Test Milks

Acid tolerance of the four *L. acidophilus* strains that were used to make unfermented acidophilus milks are as shown in Figure 2. The N1 strain appeared to be the most acid-tolerant of the four strains,

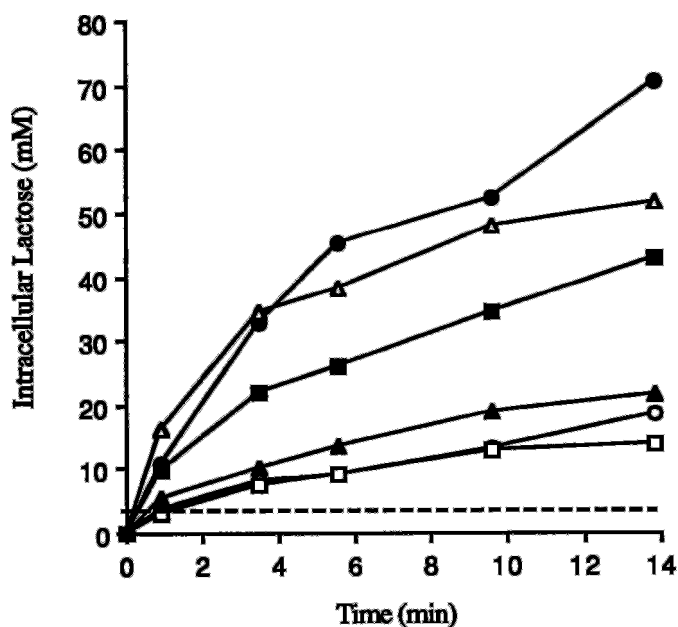


Figure 1. Lactose uptake, as measured by intracellular ^{14}C , by *Lactobacillus acidophilus* strains ATCC 4356 (●), B (Δ), Farr (■), E (▲), LA-1 (○), and N1 (□). Numbers represent means of two trials. Extracellular [^{14}C]lactose (-----) was 3 mM.

TABLE 2. Lactose content, bacterial count, and β -galactosidase (β -GAL) activity of acidophilus test milks.

Test milk	Lactose ¹	Bacterial count ²	β -GAL Activity ³
	(%)	(cfu/ml)	(U/ml)
Control	3.94 ^a	<10 est. ^b	0
4356	3.87 ^a	8.3×10^8 a	1.22 ^a
B	3.68 ^a	8.0×10^8 a	0.81 ^b
N1	3.90 ^a	8.1×10^8 a	0.50 ^c
E	4.00 ^a	1.0×10^9 a	0.79 ^b

^{a,b,c}Means within a column without a common superscript letter differ ($P < 0.05$) by Fisher's least significant difference.

¹Numbers represent means of three independent determinations of each of the three batches of milk.

²Numbers represent means of duplicate plating of each of the three batches of milk on MRS agar containing lactose. est. = Estimate.

³1 U = 1 μmol of *o*-nitrophenyl released/min. Means represent duplicate determinations of each of the three batches of milk.

surviving even after 2 h at pH 1.0. At pH 2.0, strain N1 decreased by more than 2 log cycles after 1 h and by about 3 to 4 log cycles by 2 and 3 h, respectively. Strain ATCC 4356 was equally resistant to pH 2.0, as was strain N1, decreasing only by 2 to 3 log cycles after 1, 2, and 3 h. However, no viable cells were detected at pH 1.0 after 1 h. Strains E and B were less tolerant of acid than were strains ATCC 4356 and N1. No viable cells of strain B were detected at pH 1.0 (0 h). Cells of strain E survived pH 1.0 at 0 h, but numbers were reduced to about 600/ml. Strains B and E were equally sensitive to pH 2.0. Cells of all four strains remained at fairly high concentrations even after 3 h at pH 3.0.

Breath H₂ Production and Intolerance Symptoms

Comparisons of mean hourly changes in breath H₂ production by subjects following the consumption of test meals are depicted in Figure 3. To estimate the total H₂ production, hourly breath H₂ concentrations from each subject, from 1 to 8, were summed. Total breath H₂ excretion from consumption of B, N1, or E milk was significantly lower ($P < 0.05$) than that of the control (Figure 3 and Table 3). Consumption of 4356 milk resulted in mean H₂ production that was not significantly different from those of the control and B milks.

Peak H₂ production from consumption of N1 or E milk was significantly lower ($P < 0.006$) than that of the control but not significantly different from those of 4356 or B milks. Neither 4356 nor B milk significantly reduced peak H₂ production compared with that of the control. Compared with the control, peak

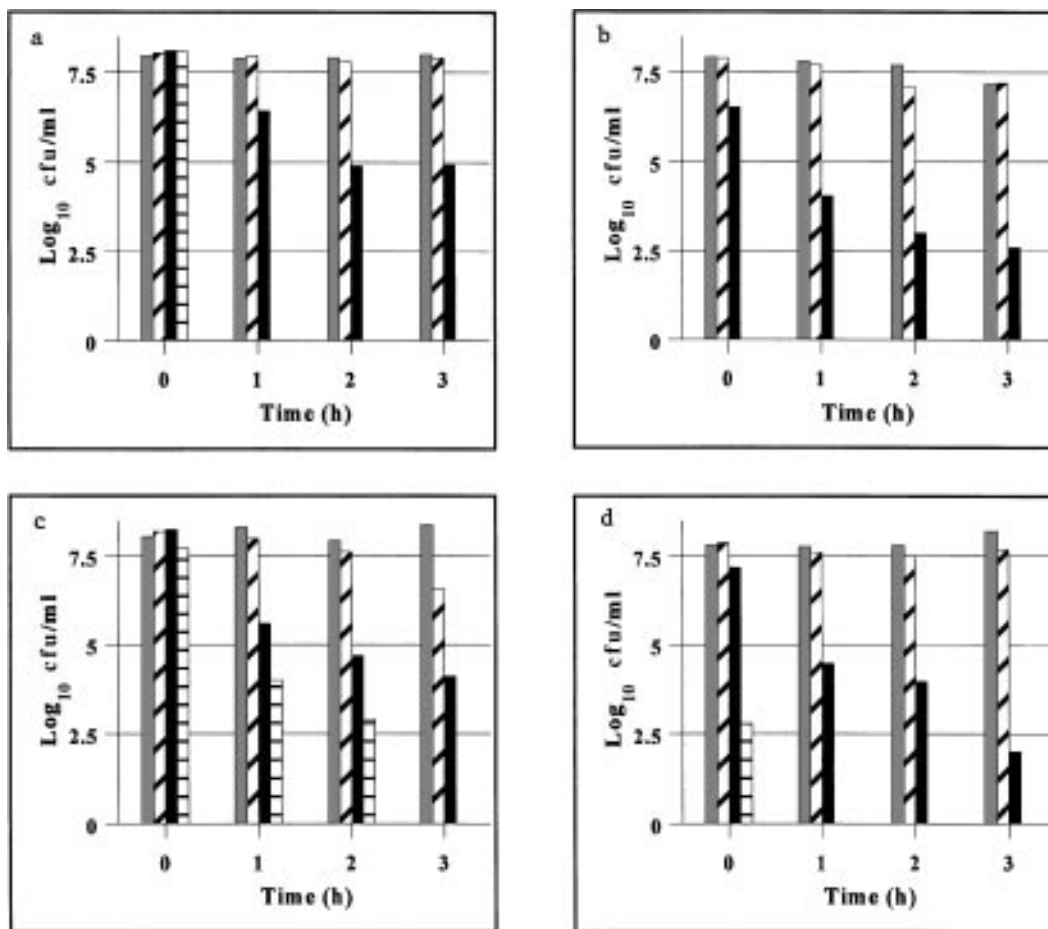


Figure 2. Survival of *Lactobacillus acidophilus* strains ATCC 4356 (a), B (b), N1 (c), and E (d) for 0 to 3 h at pH 6.7 (gray bar), 3.0 (diagonal striped bar), 2.0 (solid bar), and 1.0 (horizontal striped bar).

H₂ was delayed 1 to 2 h following consumption of 4356, N1, and E milks, but not after the consumption of B milk. All breath H₂ values dropped to near fasting concentrations by the end of the 8-h test period.

Intolerance symptoms following the consumption of test meals are summarized in Table 4. Consumption of N1 milk resulted in significantly less flatulence than did consumption of the control milk ($P < 0.05$). Significantly less bloating occurred after ingestion of E milk than after ingestion of control milk ($P < 0.05$). Diarrhea was significantly reduced with the consumption of B and N1 milks ($P < 0.05$). Abdominal rumbling was not different after consumption of the control milk or any of the four acidophilus milks.

DISCUSSION

Unfermented acidophilus milk has been thought to be potentially beneficial for alleviating lactose intoler-

ance symptoms in humans. Unfortunately, little information has been provided in earlier studies (9, 18, 20, 23, 25) regarding the culture used and the manner in which the milk had been prepared and handled. Storage at refrigeration temperature (5°C) was reported (1, 4, 5) to cause a greater loss of β -GAL activity and cell viability. Thus, the loss of β -GAL activity or viability of *L. acidophilus* cells in unfermented acidophilus milk from frozen culture addition and during refrigerated storage may have contributed to the lack of a beneficial effect of the milk on lactose maldigestion in some previous studies (23, 24, 25). Clearly, *L. acidophilus*, if grown and prepared properly before being added to milk, would contain active β -GAL, which could hydrolyze lactose in vivo. In the present study, unfermented acidophilus milk was prepared by using freshly concentrated cultures, and this milk was fed to subjects within 1 d of preparation. Preliminary studies indicated no significant

decrease in cell viability or total β -GAL activity of the milks prepared in this manner for up to 10 d at 4°C (data not shown). The NI milk, which contained low concentrations of total β -GAL, and the B and E milks, which contained intermediate concentrations of total β -GAL, significantly improved lactose digestion (Tables 2 and 3). In contrast, 4356 milk, which had the highest total β -GAL activity of the four test milks, did not significantly improve lactose digestion (Table 3). Similar to results of earlier reports (17), this observation indicated that the absolute concentration of total β -GAL activity was not the limiting factor determining improved in vivo digestion of lactose.

It has been suggested that the rate of lactose transport by the bacterial cell may be a factor limiting in vivo hydrolysis of lactose (17). However, the findings from this study did not support this theory. Consumption of milk inoculated with strain N1, which exhibited the lowest rate of lactose uptake (13.5 mM/14 min), greatly improved lactose digestion, as evidenced by decreases in breath H_2 production and intolerance symptoms (Figure 3 and Tables 3 and 4). Conversely, strain 4356, with the highest rate of lactose uptake (70 mM/14 min), did not exert a beneficial effect (Figure 3 and Tables 3 and 4). Strains B and E also transported lactose at a much lower rate than did strain 4356 but decreased total breath H_2 production more than did strain 4356 (Figure 3, Table 3).

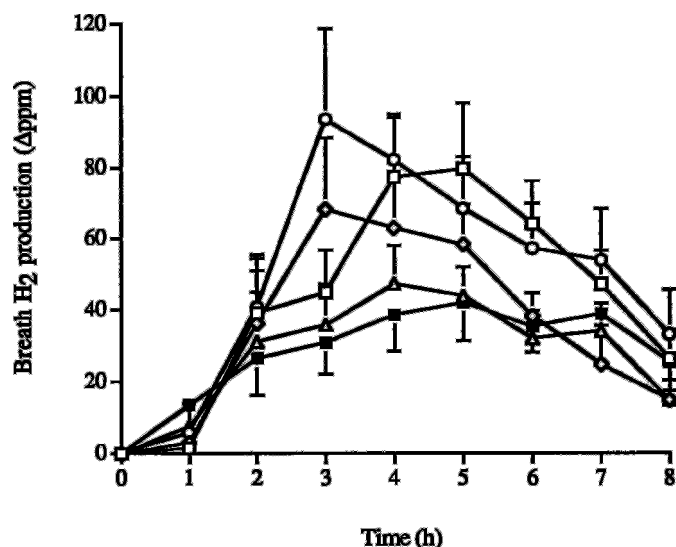


Figure 3. Corrected breath H_2 production expressed as the change, parts per million, following consumption of control milk (○), 4356 milk (□), B milk (◇), E milk (△), and N1 milk (■); unfermented test milks are designated by the acidophilus strain in the milk. Values represent means (\pm SEM) for 11 lactose maldigesting subjects.

TABLE 3. Mean values ($n = 11$) for change (Δ) in total and peak H_2 production of subjects in response to acidophilus milks during feeding trials.¹

Test milk	H_2			
	Total		Peak	
	(Δppm)			
	\bar{X}	SEM	\bar{X}	SEM
Control	434.00 ^a	69.62	127.49 ^a	21.57
4356	379.65 ^{ab}	60.15	106.72 ^{ab}	13.56
B	305.55 ^{bc}	67.33	99.84 ^{ab}	16.40
N1	245.55 ^c	43.59	71.37 ^b	11.07
E	241.20 ^c	42.95	71.00 ^b	9.16

^{a,b,c}Means within a column without a common superscript letter differ ($P < 0.05$) by Fisher's protected least significant difference.

¹The ANOVA and paired comparisons were performed by using the general linear model for a randomized block design.

Others (13, 18) have suggested that bile sensitivity of *L. acidophilus* could potentially be a limiting factor in ensuring improved in vivo lactose digestion. However, strain ATCC 4356, which was highly sensitive to bile and exhibited the highest total β -GAL activity and lactose transport rate, did not significantly improve lactose digestion or tolerance (Figure 3, Tables 3 and 4). In contrast, when compared with the control milk, strain N1, which had the lowest total β -GAL activity and the lowest lactose transport rate, but was the most bile-tolerant, resulted in a great reduction of total and peak breath H_2 excretion ($P = 0.0041$) (Figure 3 and Table 3) and significantly reduced symptoms of flatulence and diarrhea in subjects ($P < 0.05$) (Table 4). Therefore, for strains ATCC 4356 and N1, bile tolerance, but not total β -GAL activity or lactose transport, appeared to be important for improving lactose digestion in this study. This finding supports earlier reports by Noh and Gilliland (21, 22) that suggested that bacterial β -GAL does not have to be released (such as by cell lysis). Instead, increased cellular permeability, because of the presence of bile, may permit a greater amount of lactose to enter the cells and be hydrolyzed. In fact, bile was shown to increase the permeability of the bacterial cell wall but not cell lysis (21, 22). Thus, permeabilized (not lysed) cells may be necessary in order for efficient lactose hydrolysis to occur in the small intestine, which is contrary to earlier suggestions of the potential importance of cell lysis for in vivo digestion of lactose (13, 18). Previous work by McDonough et al. (18) had suggested that sonicated cells of *L. acidophilus* used in unfermented acidophilus milk may serve as an exogenous source of β -GAL in vivo. It is difficult to compare the results of this

TABLE 4. Mean (n = 11) symptom response of subjects to acidophilus milks.

Test milk	Flatulence		Bloating		Rumbling		Diarrhea	
	X	SEM	X	SEM	X	SEM	X	SEM
Control	9.93 ^a	1.73	7.44 ^a	1.80	7.81 ^a	2.06	2.69 ^a	0.76
4356	7.80 ^{ab}	1.18	5.31 ^{ab}	1.18	6.71 ^a	1.55	1.62 ^{ab}	0.63
B	8.68 ^{ab}	1.41	5.16 ^{ab}	1.20	6.48 ^a	1.22	0.46 ^b	0.27
N1	6.87 ^b	1.65	5.15 ^{ab}	1.39	6.98 ^a	2.10	1.08 ^b	0.71
E	8.40 ^{ab}	1.75	4.57 ^b	1.64	5.99 ^a	1.33	1.31 ^{ab}	0.60

^{a,b}Means within a column not sharing the same superscript letter differ ($P < 0.05$) by Fisher's protected least significant difference.

study with that of McDonough et al. (18) because the strains and storage conditions of the *L. acidophilus* were different in each study. Furthermore, no acid tolerance studies were done in the earlier work (13, 18). We speculate that the bile tolerance of the *L. acidophilus* cells in this study may allow these organisms to utilize lactose during their passage through the intestinal tract in the presence of bile, resulting in increased lactose digestion. However, further studies are necessary to determine the extent of the importance of bile tolerance of *L. acidophilus* in improving lactose digestion in vivo.

To be beneficial as a dietary adjunct, *L. acidophilus* cells must possess a certain degree of resistance to gastric acid. The tolerance of *L. acidophilus* strains to acidic conditions varies (2, 7, 11, 26). The inability of certain strains of *L. acidophilus* to survive the acidic conditions in the stomach may render them unsuitable for use as dietary supplements for lactose maldigesters. In this study, strain B was the most acid-sensitive; it was unable to survive at pH 1.0 and was reduced by 5 log cycles by pH 2.0 after 3 h (Figure 2). Strain N1 appeared to be the most acid-tolerant of the four strains tested, which, coupled with the fact that N1 was also the most bile-tolerant among the four strains, could explain the greater improvement in lactose digestion by subjects consuming N1 milk. Strain ATCC 4356 was also acid-tolerant (Figure 2) but did not exert any beneficial effect for the lactose maldigesting subjects (Figure 3 and Table 3), probably because strain ATCC 4356 was the most bile-sensitive strain used in this study.

Because inactivation of microbial lactase from sonicated cells has been shown to occur under gastric pH (16), an intact cell wall or cell membrane may function to protect the intracellular β -GAL from denaturation in the stomach. Furthermore, exposure to low pH did not affect the ability of some strains of *L. acidophilus* to adhere to human intestinal cells in tissue culture (7). Because *L. acidophilus* (such as strain N1 in this study) was also resistant to bile acids, it may contribute to in vivo lactose digestion by

surviving and potentially implanting in the intestinal tract.

In summary, an unfermented acidophilus milk can be formulated to improve lactose digestion if the cultures used are suitably selected. The main goal of this study was to increase the understanding of the important limiting factors that can be controlled during formulation of unfermented acidophilus milk. At the same time, it is acknowledged that in vivo metabolic processes involve a complicated interplay of numerous factors that extend beyond the intrinsic cellular attributes investigated in this research. At least in this study, acid and bile tolerance appear to be important aspects to consider in the selection of *L. acidophilus* strains for the purpose of aiding in vivo digestion of lactose. Further, to our knowledge, this work is the first to report on the potential limiting effects of both bile tolerance and acid tolerance of *L. acidophilus* strains in reducing lactose maldigestion. Further studies are necessary to elucidate the extent of the importance of bile and acid tolerance of *L. acidophilus* cells in in vivo lactose digestion.

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REFERENCES

- 1 Brashears, M. M., and S. E. Gilliland. 1995. Survival during frozen and subsequent refrigerated storage of *Lactobacillus acidophilus* cells as influenced by the growth phase. *J. Dairy Sci.* 78:2326.
- 2 Conway, P. L., S. L. Gorbach, and B. R. Goldin. 1987. Survival of lactic acid bacteria in the human stomach and adhesion to intestinal cells. *J. Dairy Sci.* 70:1.
- 3 Gilliland, S. E. 1985. Influence of bacterial starter cultures on nutritional value of foods: improvement of lactose digestion by consuming foods containing lactobacilli. *Cult. Dairy Prod. J.* 20: 28.
- 4 Gilliland, S. E., and R. C. Lara. 1988. Influence of storage at freezing and subsequent refrigeration temperatures on β -galactosidase activity of *Lactobacillus acidophilus*. *Appl. Environ. Microbiol.* 54:898.

- 5 Gilliland, S. E., and C. N. Rich. 1990. Stability during frozen and subsequent refrigerated storage of *Lactobacillus acidophilus* grown at different pH. *J. Dairy Sci.* 73:1187.
- 6 Gilliland, S. E., T. E. Staley, and L. J. Bush. 1984. Importance of bile tolerance of *Lactobacillus acidophilus* used as dietary adjunct. *J. Dairy Sci.* 67:3045.
- 7 Hood, S. K., and E. A. Zottola. 1988. Effect of low pH on the ability of *Lactobacillus acidophilus* to survive and adhere to human intestinal cells. *J. Food Sci.* 53:1514.
- 8 Hutkins, R. W., and C. Ponne. 1991. Lactose uptake driven by galactose efflux in *Streptococcus thermophilus*: evidence for a galactose-lactose antiporter. *Appl. Environ. Microbiol.* 57:941.
- 9 Kim, H. S., and S. E. Gilliland. 1983. *Lactobacillus acidophilus* as a dietary adjunct for milk to aid lactose digestion in humans. *J. Dairy Sci.* 66:959.
- 10 Kolars, J. C., M. D. Levitt, M. Aouji, and D. A. Savaiano. 1984. Yogurt: an autodigesting source of lactase. *New England J. Med.* 310:1.
- 11 Lankaputhra, W.E.V., and N. P. Shah. 1995. Survival of *Lactobacillus acidophilus* and *Bifidobacterium* spp. in the presence of acid and bile salts. *Cult. Dairy Prod. J.* 30:2.
- 12 Levitt, M. D., and R. M. Donaldson. 1968. Use of breath hydrogen in the study of carbohydrate malabsorption. *Clin. Res.* 16:287.
- 13 Lin, M.-Y., D. A. Savaiano, and S. K. Harlander. 1991. Influence of nonfermented dairy products containing bacterial starter cultures on lactose maldigestion in humans. *J. Dairy Sci.* 74:87.
- 14 Lin, W.-J., D. A. Savaiano, and S. K. Harlander. 1989. A method for determining β -galactosidase activity of yogurt cultures in skim milk. *J. Dairy Sci.* 72:351.
- 15 Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193:265.
- 16 Martini, M. C., G. L. Bollweg, M. D. Levitt, and D. A. Savaiano. 1987. Lactose digestion by yogurt β -galactosidase: influence of pH and microbial cell integrity. *Am. J. Clin. Nutr.* 45:432.
- 17 Martini, M. C., E. C. Lerebours, W.-H. Lin, S. K. Harlander, N. M. Berrada, J. M. Antoine, and D. A. Savaiano. 1991. Strains and species of lactic acid bacteria in fermented milks (yogurts): effect on in vivo lactose digestion. *Am. J. Clin. Nutr.* 54:1041.
- 18 McDonough, F. E., A. D. Hitchins, N. P. Wong, P. Wells, and C. E. Bodwell. 1987. Modification of sweet acidophilus milk to improve utilization by lactose-intolerant persons. *Am. J. Clin. Nutr.* 45:570.
- 19 Nannen, N. L., and R. W. Hutkins. 1991. Intracellular pH effects in lactic acid bacteria. *J. Dairy Sci.* 74:741.
- 20 Newcomer, A. D., H. S. Park, P. C. O'Brien, and D. B. McGill. 1983. Response of patients with irritable bowel syndrome and lactase deficiency using unfermented acidophilus milk. *Am. J. Clin. Nutr.* 38:257.
- 21 Noh, D. O., and S. E. Gilliland. 1993. Influence of bile on cellular integrity and β -galactosidase activity of *Lactobacillus acidophilus*. *J. Dairy Sci.* 76:1253.
- 22 Noh, D. O., and S. E. Gilliland. 1994. Influence of bile on β -galactosidase activity of component species of yogurt starter cultures. *J. Dairy Sci.* 77:3532.
- 23 Payne, D. L., J. D. Welsh, C. V. Manion, A. Tsegaye, and L. D. Herd. 1981. Effectiveness of milk products in dietary management of lactose malabsorption. *Am. J. Clin. Nutr.* 34:2711.
- 24 Reasoner, J., T. P. Maculan, A. G. Rand, and W. R. Thayer, Jr. 1981. Clinical studies with low-lactose milk. *Am. J. Clin. Nutr.* 34:54.
- 25 Savaiano, D. A., A. A. El Anouar, D. E. Smith, and M. D. Levitt. 1984. Lactose malabsorption from yogurt, pasteurized yogurt, sweet acidophilus milk, and cultured milk in lactase-deficient individuals. *Am. J. Clin. Nutr.* 40:1219.
- 26 Shah, N., and P. Jelen. 1990. Survival of lactic acid bacteria and their lactases under acidic conditions. *J. Food Sci.* 55:506.