Growth and Viability of Commercial *Bifidobacterium* spp in Skim Milk Containing Oligosaccharides and Inulin

H.-S. SHIN, J.-H. LEE, J.J. PESTKA, AND Z. USTUNOL

ABSTRACT: Two commercial strains of *Bifidobacterium* spp (Bf -1 and Bf -6) were cultured in 12% (w/w) reconstituted nonfat dry milk (NDM) containing 0, 0.5, 1.0, 3.0, or 5.0% (w/v) fructooligosaccharide (FOS), galactooligosaccharide (GOS), and inulin. Inoculated samples were incubated anaerobically at 37 °C for 48 h. Growth and activity of the cultures in the presence of FOS, GOS, and inulin were determined. Viability of each strain was assessed after 4 weeks of refrigerated storage at 4 °C. Growth promotion, enhancement of activity and retention of viability were greatest when *Bifidobacterium* Bf-1 and Bf-6 were grown in the presence of FOS followed in a descending order by GOS and inulin. The effects of oligosaccharides and inulin increased with increasing carbohydrate concentration and was maximal at 5% (w/v).

Key Words: bifidobacteria, milk, fructooligosaccharides, galactooligosaccharides, inulin, synbiotic

Introduction

OLIGOSACCHARIDES ARE A GROUP OF SHORT CHAIN NONDIgestible polysaccharides that occur naturally in foods. They are typically defined as glycosides that contain between 3 to 10 sugar moieties and are characterized by the type and sequence of the monosaccharide moieties present. They may be linear or branched (Crittenden 1999). Commercially, oligosaccharides are produced by enzymic processes either by hydrolysis of polysaccharides or synthesis from smaller sugars using transglycosylases; however, the products produced commercially for food application are not chemically homogeneous but rather are mixtures containing oligosaccharides with different numbers of monosaccharide moieties and different glycosidic linkages (Crittenden and Playne 1996). Initially, oligosaccharides were developed as sucrose substitutes and for use as bulking agents in foods (Crittenden 1999). Later, it was determined that certain oligosaccharides had the potential to increase bifidobacteria in the colon without being utilized by other intestinal bacteria (Ballongue and others 1997; Bouhnik and others 1997; Alles and others 1996; Gibson and others 1995; Hayakawa and others 1990; Hidaka and others 1986). Because of their prebiotic properties oligosaccharides have received much recent attention as functional food ingredients (Playne and Crittenden 1996). The ability of oligosaccharides to act as prebiotics has been used as a marketing tool for these products. In addition, it has promoted research into the ability of oligosaccharides to provide beneficial changes in the composition and metabolism of the colonic microflora (Gibson and Roberfroid 1995). The mechanisms by which prebiotics may provide health benefits remain unclear.

Currently, there are 9 types of oligosaccharides commercially produced, all claimed to be bifidogenic by the manufacturers (Crittenden 1999). Fructooligosaccharides (FOS), galactooligosaccharides (GOS,) and transgalactosyloligosaccharides, TOS), and soybean oligosaccharides (main functional components being raffinose and stachyose) have been most extensively studied and may provide the best evidence of prebiotic effects in humans (Crittenden 1999; Crittenden and Playne 1996). Inulin is used primarily as a fat substitute (Gorski 1995). Similar to other oligosaccharides, inulin is not hydrolyzed or absorbed in the small intestine and has been shown to reach the colon mostly intact and is thought to act as a prebiotic (Roberfroid and others 1998; Kleesen and others 1997; Gibson and others 1995). Inulin is heterogeneous with respect to polymer chain length. Its degree of polymerization (DP) ranges 3 to 60, but it primarily consists of DP 20-25 (Van Loo and others 1995).

Bifidobacteria have been associated with health-promoting effects (Sanders 1993), and thus have been incorporated into fermented dairy foods and other dairy foods (Tannock 1999). The ultimate intent of this strategy is to provide the gastrointestinal (GI) tract of humans with elevated viable populations of bifidobacteria. Thus, viability of bifidobacteria in dairy products has received much attention (Shin and others 2000; Dave and Shah 1997; 1998; Ishibashi and Shimamura 1993; Hekmat and McMahon 1992). Although the effects of oligosaccharides on colonic bifidobacteria have been investigated, there are no reports on the effects of oligosaccharides on bifidobacteria stored in dairy foods.

The concept of synbiotics (mixture of pro- and prebiotics which in synergy provide health benefits to the host) (Gibson and Roberfroid 1995) has not been as extensively tested. Such an approach could ultimately provide for improved viability of bifidobacteria in food products with perhaps prolonged shelf-life, an increased number of ingested bacteria reaching the colon in a viable form, and stimulation of bifidobacteria (both exogenous and endogenous) in the colon. Therefore, the purpose of this study was to investigate the effect of commercially sold oligosaccharides and inulin on growth, activity, and viability of commercial strains of probiotic *Bifidobacterium* spp. that are used in manufacture of dairy products.

Results and Discussion

TABLE 1 SHOWS THE MEAN DOUBLING TIME OF EACH COMMERcial *Bifidobacterium* spp. grown in skim milk in the presence of FOS, GOS, and inulin at concentrations 0.5%, 1.0%, 3.0%, and 5%. Mean doubling time was used as a measure of the efficacy of various carbon sources in modulating growth rate. Growth promotion of *Bifidobacterium* spp. by oligosaccharides and inulin was obtained dose dependently over the range 0% to 5% as evi-

Table 1—Doubling time of commercial *Bifidobacterium* spp. in skim milk containing oligosaccharides and inulin.

Treatments	Mean doubling time[1] (min)		
	CHO level (%)	Bf-1	Bf-6
Control	0	237±10	242±13
Fructooligosaccharide (FOS)	0.5	215±16	227±10
	1.0	200±12*	196±15*
	3.0	166±01*	171±08*
	5.0	132±15*	125±10*
Galactooligosaccharide (GOS)	0.5	218±08	225±12
	1.0	213±10	221±17
	3.0	184±12*	189±06*
	5.0	148±05*	140±17*
Inulin	0.5	230±07	234±10
	1.0	217±19	226±12
	3.0	211±20	220±18
	5.0	179±14*	170±15*

[1]Mean doubling time (T_d) = ln2/μ (specific growth rate); $\mu = \ln X_2 - \ln X_1 / t_2 - t_1$. Means ± standard deviations; n=3 for all treatments.
*Indicates significantly different ($p < 0.05$) from the control (comparisons are made only with the control).

Table 2—Acetic and lactic acid production by commercial *Bifidobacterium* spp. grown in skim milk containing 5% oligosaccharides and inulin.

Treatments[1]	Bf-1			Bf-6		
	Acetic acid (mM)	Lactic acid (mM)	Ratio[2]	Acetic acid (mM)	Lactic acid (mM)	Ratio
(control)	6.2 ± 0.5[a]	4.9 ± 0.5[a]	1.3[a]	10.3 ± 1.0[a]	5.7 ± 0.4[a]	1.8[a]
FOS	28.7 ± 4.8[b]	15.5 ± 0.9[c]	1.9[b]	15.8 ± 2.6[b]	11.6 ± 1.2[b]	1.4[b]
GOS	10.9 ± 1.6[c]	7.0 ± 0.9[b]	1.6[c]	14.2 ± 0.5[b]	11.7 ± 3.8[b]	1.3[b]
Inulin	8.2 ± 0.5[d]	5.5 ± 0.2[a]	1.5[a]	11.3 ± 0.8[a]	7.5 ± 2.2[a]	1.2[b]

[1]FOS= Fructooligosaccharide; GOS=Galactooligosaccharide; FOS, GOS and inulin.
[2] acetic acid/lactic acid
[a-d]Means with different superscripts are significantly different ($p < 0.05$). Comparisons are made only within the same column. Means ± standard deviations; n=3 for all treatments.

denced by decreased mean doubling time with increased concentration of FOS, GOS, or inulin, indicating that both strains grew faster in the presence of these carbohydrates compared to the controls.

Among the carbohydrate sources tested, FOS was the most effective in enhancing growth rate of both *Bifidobacterium* Bf-1 and Bf-6 in skim milk. At concentrations of > 1% mean doubling time for both strains was significantly reduced ($p < 0.05$) compared to the controls. For both strains a 5% FOS concentration provided the shortest mean doubling times (132 and 125 min, respectively) among the carbohydrate sources and concentrations tested. These results are consistent with previous reports on the ability of FOS to stimulate the proliferation of bifidobacteria relative to other intestinal microflora in vitro culture models simulating the colon (Gibson and Wang 1994), stimulation of bifidobacteria in germfree rats inoculated with a human fecal flora (Djouzi and Andrieux 1997) and selective stimulation of bifidobacteria in human colon by FOS in human-feeding trials (Gibson and others 1995; Roberfroid and others 1998). Dubey and Mistry (1996) reported that maximal counts and generation times of *Bifidobacterium breve, B. bifidum, B. infantis, B. longum* (obtained from ATCC) were not influenced by 0.5% FOS when these organisms were grown in infant formula (hydrolyzed casein- or soy-based), but they did not investigate concentrations of FOS above 0.5%. Consistent with their findings, we did not observe significant differences (compared to the control) in mean doubling times when either *Bifidobacterium* Bf-1 or Bf-6 was grown in the presence of 0.5% FOS (Table 1).

In accordance with growth stimulation, both acetic and lactic acid production by *Bifidobacterium* Bf-1 and Bf-6 were also enhanced ($p < 0.05$) by the presence of FOS in skim milk compared to the controls (Table 2). Production of acetic and lactic acids by *Bifidobacterium* Bf-1 was higher (28.7 and 15.5 mM, respectively) compared to Bf-6 which produced 15.8 and 11.6 mM of acetic and lactic acids, respectively. Acetic and lactic acid production by *Bifidobacterium* Bf-1 and Bf-6 controls were 6.2, 4.9 mM and 10.3, 5.7 mM, respectively. Molar ratios of acetic to lactic acid was also calculated and are presented in Table 2. The bifidobacteria fermentation pathway results in 3 moles of acetic acid and 2 moles of lactic acid per 2 moles of glucose in an ideal synthetic medium. Thus, yielding a theoretical molar ratio (acetic:lactic) of 1.5 (Scardovi and Trovelli 1965). Although lactic acid production is desirable in fermented dairy foods, a high concentration of acetic acid can result in distinct vinegar flavor in products. Thus, a high acetic to lactic acid ratio is typically undesirable in fermented dairy foods. The overall molar ratios of acetic to lactic acid we calculated were similar to the theoretical molar ratio.

Although, GOS also stimulated the growth of *Bifidobacterium* Bf- 1 and Bf-6 when added to skim milk, it was not as effective as FOS. A > 3% concentration of GOS was needed to stimulate ($p < 0.05$) the growth of both strains as evidenced by mean doubling times (Table 1). Fermentation of GOS by colonic bifidobacteria and increased fecal numbers of bifidobacteria in rats associated with a human fecal microflora was reported by Rawland and Tanaka (1993). The ingestion of GOS has also been demonstrated to promote increased fecal bifidobacteria numbers in human feeding trials (Bouhnik and others 1997; Ito and others 1990). Consistent with the data on growth, the activities of both *Bifidobacterium* Bf-1 and Bf-6 were also enhanced ($p < 0.05$) by GOS compared to the control, however, both acetic and lactic acid production (10.9 and 7.0 mM) by Bf-1 in the presence of GOS was less ($p < 0.05$) than that produced by this organism when it was grown in the presence of FOS. In case of Bf-6, acetic and lactic acid production was similar both with FOS and GOS.

Inulin was the least effective in stimulating the growth of *Bifidobacterium* Bf-1 and Bf-6 among the carbohydrate sources tested as evidenced by the mean doubling times (Table 1). A 5% concentration of inulin was needed to observe significant differences ($p < 0.05$) in mean doubling times compared to controls. This was also apparent with acetic and lactic acid production which was lower ($p < 0.05$) when either strain was grown in the presence of inulin. Although, they were slightly higher than the controls. Roberfroid and others (1998) reported in vitro fermentation of inulin by human fecal bacteria, molecules with DP > 10 were fermented on the average half as quickly as molecules with DP < 10. In similar in vitro experiments Hopkins and others (1998) reported that GOS and FOS having lower DP were best in supporting growth of bifidobacteria (obtained from National Collection of Food Bacteria, Reading, U.K.). In contrast, carbohydrates with high DP were poor bifidobacterial substrates. Very little is know about the mechanism of carbohydrate uptake by bifidobacteria; however, it appears likely that the substrate transport systems may be more efficient for dimeric and oligomeric carbohydrate sources.

Table 3 shows the viability of commercial *Bifidobacterium* Bf-1 and Bf-6 grown in skim milk containing oligosaccharides and inulin after 4 weeks of refrigerated storage at 4 °C. Because growth of bifidobacteria was significantly affected by the type of oligosaccharide and inulin, the initial viable cell counts varied prior to refrigerated storage (immediately after 48h cultivation with or without oligosaccharides and inulin). Percent viability was calculated and reported. Viability of both strains of bifidobacteria was reduced ($p < 0.05$) for all treatments after 4 wk of refrigerated storage; however, viabilities were higher for both strains when

Table 3—Viability of commercial *Bifidobacterium* spp. grown in skim milk containing oligosaccharides and inulin after 4 wk of refrigerated storage at 4 °C.

		% Viability[1]	
Treatments	**CHO level (%)**	**Bf-1**	**Bf-6**
Control	0	11.6 ± 1.6[a]	9.3 ± 1.7[a]
Fructooligosaccharide (FOS)	0.5	16.4 ± 4.3[a]	10.1 ± 0.4[a]
	1.0	17.5 ± 2.6[b]	13.4 ± 2.5[a]
	3.0	41.6 ± 3.7[c]	28.7 ± 3.9[c]
	5.0	67.3 ± 7.4[d]	44.6 ± 3.9[d]
Galactooligosaccharide (GOS)	0.5	14.1 ± 1.3[a]	9.7 ± 1.8[a]
	1.0	15.4 ± 2.5[b]	11.5 ± 1.9[b]
	3.0	32.1 ± 2.2[c]	24.9 ± 3.2[c]
	5.0	52.2 ± 4.8[d]	39.3 ± 3.5[d]
Inulin	0.5	12.1 ± 2.3[a]	9.5 ± 1.8[a]
	1.0	14.3 ± 2.6[a]	10.2 ± 2.7[a]
	3.0	17.9 ± 5.5[a]	14.6 ± 3.9[a]
	5.0	31.5 ± 4.8[b]	18.9 ± 5.2[a]

[1] % viability =(CFU after 4 weeks storage/initial CFU)x100.
[a-c] Means with different superscripts are significantly different ($p < 0.05$). Comparisons are made only within the same column. Means ± standard deviations; n=3 for all treatments.

they were grown and stored in the presence of oligosaccharides and inulin compared to the controls. Increased viability was observed dose dependently over the range from 0 to 5%. Best retention of viability was observed when cultures were grown in the presence of 5% FOS or GOS. A 67% viability of Bf-1 and 45% viability of Bf- 6 were retained after 4 weeks of refrigerated storage when these cultures were grown in the presence of 5% FOS. A 52 and 39% retention of viability was observed when Bf-1 and Bf-6, respectively were grown in the presence of 5% GOS. Inulin was the least effective ($p < 0.05$) in retaining viability of either strain.

Loss of viability of bifidobacteria is typically more pronounced in fermented milk than in unfermented milk (Hughes and Hoover 1995; Yaguchi 1984) due to the acid injury to the organism. Lankaputhra and others (1996) observed that viability of *B. infantis* in 12% skim milk at pH 4.3 was decreased by 30% after 12 d of storage at 4 °C. After 24 d at the same temperature the counts decreased by more than 82%. Medina and Jordano (1994) reported on bifidobacterial counts of fermented milk produced in Spain stored at 7 °C. They observed a 93% decrease in the bifidobacterial population past the product expiration date. In our study, 67 to 39% of the viability was retained after 28 d of storage at 4 °C which is much higher than previous reports. Modler and others (1990) and Hayes and others (1996) observed no differences in growth and viability of bifidobacteria due to FOS that was added during manufacturing of ice-cream (Modler and others 1990) and Edam cheese (Hayes and others 1996); however, in both of these studies FOS was merely added and not provided as a substrate for bifidobacteria.

Conclusion

THE DEGREE OF ENHANCEMENT OF GROWTH, ACTIVITY, AND VIability of *Bifidobacterium* Bf-1 and Bf-6 in skim milk were dependent on the carbon source and concentration as well the strain of *Bifidobacterium*; however, further studies are needed with additional strains of commercial bifidobacteria used as dietary adjuncts to optimize their growth and viability in commercial dairy products. Increased health benefits of synbiotics still need to be investigated.

Materials and Methods

Culture preparation

Commercial strains *Bifidobacterium bifidum* (Bf-1 and Bf-6) were obtained from Sanofi Bio-Industries (Waukesha, Wis., U.S.A.). Each culture was cultured and subcultured anaerobically in MRS medium (Difco, Detroit, Mich., U.S.A.) (De Man and others 1960) containing 5% (w/v) lactose (MRSL) at 37 °C for 48 h using Gas Paks (Becton Dickinson Co., Cockeysville, Md., U.S.A.). Cultures were centrifuged 15 min at 1000 × g at 4 °C and resuspended in 12% w/v pasteurized (63 °C, 30 min) nonfat dry milk (NDM; Difco) (approximately 10^8 CFU/ml).

Growth of bifidobacteria in the presence of oligosaccharides and inulin

Commercially available fructooligosaccharides (FOS; 95% FOS, 5% glucose, fructose and sucrose), inulin (99%) (Rhône-Poulenc Inc., Cranbury, N.J., U.S.A.) and galactooligosaccharide (GOS; > 50%) (Samyang Genex Co., Ltd., Seoul, Korea) were added at a 0.5%, 1%, 3%, and 5% (w/v) to 12% (w/v) reconstituted nonfat dry milk (NDM) and pasteurized at 70 °C for 15 min. Controls were devoid of oligosaccharides or inulin. Duplicate tubes of each treatment were prepared. Pasteurized tubes were inoculated with either *Bifidobacterium* Bf-1 or Bf-6 at 5% (v/v). Inoculated tubes were incubated anaerobically at 37 °C for 48 h using Gas Paks (Becton Dickinson Co.). An aliquot from each sample was taken at appropriate intervals and diluted (1:10, v/v) with 0.2% (w/v) EDTA, (pH 12.0) and turbidity was measured at 640 nm (Hughes and Hoover 1995). Uninoculated reconstituted and pasteurized NDM was diluted with 0.2% (w/v) EDTA and used as the blank. Specific growth rate (μ) for each culture was calculated using the following equation: $\mu = (\ln X_2 - \ln X_1) / (t_1 - t_2)$, where X_2 and X_1 are the cell density at time t_2 and t_1. Mean doubling time (T_d) was calculated as : $T_d = \ln 2 / \mu$. Another sample was collected for pH measurements.

Activity determination

Culture activity in the presence of different oligosaccharides and inulin was determined by measuring end products of fermentation (lactic and acetic acid) using HPLC. NDM containing 5% FOS, GOS and inulin and fermented with *Bifidobacterium* Bf-1 or Bf-6 as described previously were prepared for HPLC analysis using the method described by Dubey and Mistry (1996). One-hundred microliters of 15.8 N HNO_3 and 14.9 ml of 0.009 N H_2SO_4 were added to 1.5 ml of sample and centrifuged at 5000 × g for 10 min. The supernatant was filtered using Whatman #1 filter paper, 0.22 μm membrane filter (Millipore Corp., Bedford, Mass., U.S.A.), eluted through a reverse-phase Supelclean tube (Supelco Inc., Bellefonte, Penna.) and stored in HPLC vials at −20 °C until HPLC analysis. The HPLC system (Waters Associates Inc., Milford, Mass., U.S.A.) consisted of a M-45 solvent delivery system, a 486 UV/Vis tunable absorbance detector and a 730 data module. An Aminex HPX-87H column (300 mm × 7.8 mm, Bio-Rad Laboratories, Richmond, Calif., U.S.A.) and a guard column with disposable cartridges H^+ (Bio-Rad Laboratories) maintained at 65 °C was used for the analysis. The degassed mobile phase of 0.009 N H_2SO_4 filtered through a 0.45 μm membrane filter (Millipore Corp.) was used at a flow rate of 0.6 ml/min. The wavelength of detection was optimized at 220 nm for the organic acids being quantitated (Bouzas and others 1991). Standard solutions of organic acids (lactic and acetic acid; Sigma, St. Louis, Mo., U.S.A.) were prepared to establish elution times and calibration curves.

Determining viability during refrigerated storage

In a separate experiment, *Bifidobacterium* Bf-1 and Bf-6

were cultured anaerobically at 37 °C for 48h with 0.5%, 1.0%, 3.0%, or 5.0 % (w/v) oligosaccharides and inulin as described previously. Controls had no oligosaccharide or inulin added. All samples were stored at 4.0 ± 1 °C for 4 wk. One ml of each fermented milk sample was diluted with 99 ml of sterile 0.1% (w/v) peptone (Difco) and subsequent serial dilutions were made. Bifidobacteria were enumerated using MRSL containing 1.5% Bacto agar. The inoculated plates were incubated anaerobically at 37 °C for 48h using Gas Paks (Becton Dickinson Co). The colonies were counted using a Quebec colony counter (Fisher Scientific, Pittsburgh, Pa., U.S.A.). Percent viability of each culture in the presence of different oligosaccharides and inulin was calculated as follows:

$$\% \text{ viability} = (\text{CFU at 4 weeks of storage}/\text{initial CFU}) \times 100.$$

Statistical analysis

Each experiment was independently replicated three times in a completely randomized design. All analyses and platings were done in triplicate. Statistical analysis was conducted using Sigma Stat 2.0 (Jandel Corp., San Rafael, Calif. U.S.A.). Comparisons were made using Student-Newman-Keuls test for multiple comparisons. A $p < 0.05$ was considered statistically significant.

References

Alles MS, J Hautvast FM, Nagengast R, Hertemink MJ, van Lacre (INIT/), Jansen J. 1996. Fate of fructo-oligosaccharides in the human intestine. Br. J. Nutr. 76:211-221.

Ballongue J, Schumann C, Quignon P. 1997. Effects of lactulose and lactitol on colonic microflora and enzymatic activity. Scand. J. Gastroenterol. 32:Suppl. 222:41-44.

Bouhnik Y, Flourie BL, D'Agay-Abensour P. Pochart G, Gramet M, Durand M, Rambaud JC. 1997. Administration of transgalactooligosaccharides increases fecal bifidobacteria and modifies colonic fermentation metabolism in healthy humans. J. Nutr. 127: 444-448.

Bouzas J, Kantt CA, Bodyfelt F, Torres JA. 1991. Simultaneous detections of sugars and organic acids in cheddar cheese by high pressure liquid chromatography. J. Food Sci. 56:276-279.

Crittenden R. 1999. Prebiotics. In: Tannock J, editor. Probiotics: A critical review. Norfolk, England: Horizon Scientific Press. p 141-156.

Crittenden RG, Playne MJ. 1996. Production, properties, and applications of food-grade oligosaccharides. Trends Food Sci. Technol. 7: 353-361.

Dave RI, Shahn NP. 1997. Effect of level of starter culture on viability of yoghurt and probiotic bacteria in yoghurts. Food Australia 49:164-168.

Dave RI, Shah NP. 1998. Ingredient supplementation effects on viability of probiotic bacteria in yogurt. J. Dairy Sci. 81:2804-2816.

de Man JC, Rogosa M, Sharpe ME. 1960. A medium for the cultivation of *Lactobacilli*. J. Appl. Bacteriol. 23:130-135.

Djouzi Z, Andrieux C. 1997. Compared effects of three oligosaccharides on metabolism of intestinal microflora in rats inoculated with a human faecal flora. Br. J. Nutr. 78:313-324.

Dubey UK, Mistry VV. 1996. Effect of bifidogenic factors on growth characteristics of bifidobacteria in infant formula. J. Dairy Sci. 79:1156-1163.

Gibson GR, Roberfroid MB. 1995. Dietary modulation of the human colonic microbiota—Introducing the concept of prebiotics. J. Nutr. 125: 1401-1412.

Gibson GR, Wang X. 1994. Enrichment of bifidobacteria from human gut contents by oligofructose using continuous culture. FEMS Microbiol. Lett. 118:121-128.

Gibson GR, Beatty ER,Wang X, Cummings JH. 1995. Selective stimulation of bifidobacteria in the human colon by oligofructose and inulin. Gastroenterology 108: 975-982.

Gorski D. 1995. Fat replacement technologies. Dairy Foods 1:38-39.

Hayakawa K, Mizutani J, Wada K, Masai T, Yoshihara I, Mitsuoka T. 1990. Effects of soybean oligosaccharides on human faecal microflora. Microb. Ecol. Health Dis. 3: 293-303.

Hayes W, Feijoo S, Martin J. 1996. Effect of lactulose and oligosaccharides on survival of *Bifidobacterium longum* in Edam cheese. J. Dairy Sci. 79(Suppl.1):118.

Hekmat S, McMahon DJ. 1992. Survival of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in ice-cream for use as probiotic food. J. Dairy Sci. 75:1415-1422.

Hidaka H, Eida T, Takizawa T, Tokunaga T, Tashira Y. 1986. Effects of fructooligosaccharides on intestinal flora and human health. Bifidobacteria Microflora 5:37-50.

Hopkins MJ, Cummings JH, Mcfarlane GT. 1998. Inter-species differences in maximum specific growth rates and cell yields of bifidobacteria cultured on oligosaccharides and other simple carbohydrate sources. J. Appl. Microb. 85:381-386.

Hughes DB, Hoover DG. 1995. Viability and enzymic activity of bifidobacteria in milk. J. Dairy Sci. 78:268-276.

Ishibashi, Shimamura S. 1993. Bifidobacteria: Research and development in Japan. Food Technol. 47(6):126-135.

Ito M, Deguchi Y, Miyamori A, Matsumoto K, Kikuchi H, Kobayashi Y, Yajiama T, Kan T. 1990. Effects of administration of galactooligosaccharides on the human fecal microflora, stool weight and abdominal sensation. Microb. Ecol. Health Dis. 3:285-292.

Kleesen B., Sykura B, Zunft HJ, Blaut M. 1997. Effects of inulin and lactose on fecal microflora, microbial activity, and bowel habit in elderly constipated persons. Am. J. Clin. Nutr. 65: 1397-1402.

Lankaputhra WE, Shah NP, Britz ML. 1996. Survival of bifidobacteria during refrigerated storage in the presence of acid and hydrogen peroxide. Milchwissenschaft 51:65-70.

Medina LM, Jordano R. 1994. Survival of constitutive microflora in commercially fermented milk containing bifidobacteria during refrigerated storage. J. Food Prot. 56:731-733.

Modler W, McKellar R, Goff H, Mackie D.. 1990. Using ice-cream as a mechanism to incorporate bifidobacteria and fructooligosaccharides into the human diet. Cultured Dairy Prod. J. 25(8):4-9.

Playne MJ, Crittenden R. 1996. Commercially available oligosaccharides. Bull. Int. Dairy Fed. 313:10-22.

Rawland IR, Tanaka R. 1993. The effect of transglycosylated oligosaccharides on gut flora metabolism in rats associated with a human faecal microflora. J. Appl. Bacteriol. 74:667-674.

Roberfroid MB, Van Loo JAE, Gibson GR. 1998. The bifidogenic nature of chicory inulin and its hydrolysis products. J. Nutr. 128:11-19.

Sanders, ME. 1993. Effect of consumption of lactic cultures on human health. In: Kinsella J, editor. Advances in Food and Nutrition Research. San Diego, Calif: Academic Press. p 67-130.

Scardovi V, Trovatelli LD. 1965. The fructose-6-phosphate shunt as a peculiar pattern of hexose degradation in the genus *Bifidobacterium*. Ann. Microbiol. Enzymol. 15:19-27.

Tannock,J ,editor. 1999. Probiotics: A critical review. Norfolk, England. Horizon Scientific Press. p 57-62.

Shin HS, Lee JH, Pestka J, Ustunol Z. 2000. Viability of bifidobacteria in commercial dairy products during refrigerated storage. J. Food Prot. 63:327-331.

Van Loo JP, Coussement P, De Leenheer L, Hoebregs H, Smits G. 1995. On the presence of inulin and oligofructose as natural ingredients in the western diet. Crit. Rev. Food Sci. Nutr. 35(6):525-552.

Yaguchi H. 1984. The progress of milk products containing viable bifidobacteria. Jpn. J. Dairy Food Sci. 33:A203-A212.

MS 19990928 received 9/20/99; revised 3/22/00; accepted 4/10/00.

State of Michigan Animal Industries Initiative and Michigan Agricultural Experiment Station is acknowledged for partial support of this research.

Authors are with the Department of Food Science and Human Nutrition, Michigan State University, E. Lansing MI 48824-1224. Send correspondence to Z. Ustunol (E-mail: ustunol@msu.edu).