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Effect of Oligosaccharides and Inulin on the Growth and Viability of Bifidobacteria in Skim Milk

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Abstract The effects of food grade fructooligosaccharide (FOS), isomaltooligosaccharide (MOS), galactooligosaccharide (GOS), and inulin on the growth of five strains of bifidobacteria in fermented milk were investigated. Their effect on culture viability during refrigerated storage was also determined. FOS showed the highest growth-promoting activity for all bifidobacteria except for *Bifidobacterium bifidum*. Growth rates of *B. adolescentis*, *B. breve*, and *B. infantis* were stimulated by oligosaccharides and inulin, whereas *B. longum* growth was stimulated by the oligosaccharides but not inulin. In contrast, growth of *B. bifidum* was enhanced only by inulin. Both acetic and lactic acid production by *Bifidobacterium* spp. was also enhanced in the presence of 5.0% oligosaccharides. The viability of bifidobacteria cultured with oligosaccharides and inulin, particularly with FOS, was significantly higher than control cultures after 4 weeks of refrigerated storage. The utilization of oligosaccharides is likely to enhance the growth rate, activity, and viability of bifidobacteria.

Keywords: bifidobacteria, growth, viability, oligosaccharides, inulin, probiotic

Introduction

Bifidobacteria are Gram-positive, anaerobic, non-spore forming rod-shaped bacteria, and one of the predominant groups of microorganisms in the intestine of healthy infants and adults. Since Metchnikoff (1) proposed that the consumption of fermented dairy products resulted in improved health and a longer life, there have been many reports showing that bifidobacteria have a variety of beneficial and health-promoting effects. These include preventing colonization of the intestine by pathogens (2), eliminating constipation (3), and improving lactose utilization (4). Furthermore, in recent years a number of studies have shown that bifidobacteria can play an important role in modulating various immunological functions (5-7). For these reasons, there has been an increased interest in incorporating bifidobacteria into fermented dairy foods and other dairy foods to enhance their health effects (8). Bifidobacteria used for the production of fermented milks are selected on the basis of their growth potential in milk as well as the origin of the strains. However, bifidobacteria are nutritionally fastidious and many strains are oxygen and pH sensitive and grow poorly in milk (9, 10). Thus, there are many questions regarding how to incorporate these microorganisms and maintain their viability in fermented milks.

Trace elements (11) and vitamins (12) have been studied as nutrients to enhance the growth of bifidobacteria. Additional studies have focused on finding bifidogenic factors that are selectively utilized by bifidobacteria. These factors include components found in human milk such as *N*-acetyl glucosamine (13), porcine mucine (14), and human casein hydrolysates (15) which are deficient in cow's milk. Yazawa *et al.* (16) reported on various indigestible saccharides useful for increasing the number of intestinal bifidobacteria. Benno *et al.* (17) reported a significant increase in human fecal bifidobacteria by the ingestion of raffinose obtained from beet molasses. Recently, fructooligosaccharide (FOS) (18, 19), *trans*-galactosyl oligosaccharides (20), isomaltooligosaccharide (MOS) (21), 4'-galactosyl-lactose (22), and other oligosaccharides have been reported as effective bifidogenic factors resulting in the proliferation of human intestinal bifidobacteria.

Oligosaccharides are a group of short chain non-digestible polysaccharides that usually occur naturally in foods (23). Oligosaccharides are not hydrolyzed or absorbed in the small intestine and have been shown to reach the colon mostly intact and are thought to act as a prebiotic (24-27). Among the oligosaccharides, FOS, which consists of 1-kestose, nystose, and 1^F-β-fructofuranosyl nytrose, galactooligosaccharide (GOS), which is a polymer containing 1 to 4 molecules of galactose, and MOS, which consists of isomaltose and panose, are commercially available (23). Also commercially available, inulin is a widespread carbohydrate belonging to the fructan group, a mixture of fructose chains with a degree of polymerization (DP) varying from 3-60 that has a DP value of 20-25 (28). The concept of synbiotics (mixture of pro- and prebiotics which in synergy provide health benefits to the host) (29) has not been fully investigated. The ultimate intent of the synbiotic strategy is to provide the gastrointestinal tract of humans with viable populations of bifidobacteria and to stimulate the growth of bifidobacteria (both exogenous and endogenous) in the colon. Therefore, the purpose of this study was to investigate the effect of food grade oligosaccharides (FOS, GOS, and MOS) and inulin on the growth, activity, and viability of five *Bifidobacterium* strains in skim milk.

Materials and Methods

Culture preparation Five strains of bifidobacteria, *B.*

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adolescentis 3352, *B. bifidum* 3418, *B. breve* 3220, *B. infantis* 3368, and *B. longum* 3128 were purchased from the Korean Collection for Type Cultures (Daejeon, Korea). Each strain was cultured and subcultured anaerobically in MRS medium (Difco, Detroit, MI, USA) containing 5% (w/v) lactose (MRSL) at 37°C for 48 hr using Gas Paks (Becton Dickinson Co., Cockeysville, MD, USA). 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 fructooligosaccharudes (FOS, >41%; glucose and sucrose, >33%) were obtained from CJ Corporation (Seoul, Korea). Galactooligosaccharide (GOS, >50%) and isomaltooligosaccharide (MOS, >58%) were obtained from Corn Products International Inc. (Westchester, IL, USA). Inulin (99%) was obtained from Orafti Active Food Ingredients (Malvern, PA, USA). Each oligosaccharide was added at a concentration of 0.1-5.0 to 12%(w/v) reconstituted NDM and pasteurized at 70°C for 15 min. The control sample was devoid of oligosaccharides or inulin. Duplicated culture tubes were prepared for each treatment. Pasteurized culture tubes were inoculated with *B. adolescentis* 3352, *B. bifidum* 3418, *B. breve* 3220, *B. infantis* 3368, and *B. longum* 3128 at 5%(v/v). Inoculated culture tubes were incubated anaerobically at 37°C for 48 hr using Gas Paks (Becton Dickinson Co.). Aliquots from each sample were 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. Uninoculated, reconstituted and pasteurized NDM was diluted with 0.2% (w/v) EDTA and used as the blank. The 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 densities at times t_2 and t_1. Mean doubling time (T_d) was calculated as: $Td = \ln 2/\mu$.

Bifidobacterial activity determination Culture activity in the presence of different oligosaccharides and inulin was determined by measuring the end products of fermentation (lactic and acetic acid) using HPLC. NDM samples containing 5.0% FOS, GOS, MOS, and inulin, and fermented with *B. adolescentis* 3352, *B. bifidum* 3418, *B. breve* 3220, *B. infantis* 3368, and *B. longum* 3128 as described previously were prepared for HPLC analysis using the methods described by Dubey and Mistry (30). 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 5,000×g for 10 min. The supernatant was filtered using Whatman #1 filter paper and a 0.22 μm membrane filter (Millipore Corp., Bedford, MA, USA), eluted through a reverse-phase Supelclean tube (Supelco Inc., Bellefonte, PA, USA), and stored in HPLC vials at -20°C until HPLC analysis. The HPLC system (Waters Associates inc., Milford, MA, USA) consisted of a Chem Station (Agilent Technologies, Palo Alto, CA, USA) with a diode array detector (Model 1100; Agilent Technologies). An Aminex HPX-87H column (300×7.8 mm, Bio-Rad Laboratories, Richmond, CA, USA) and a guard column with disposable H^+ cartridges (Bio-Rad Laboratories) maintained at 65°C were used for the analysis. The identities of the peaks were established by comparing retention times of the peaks with those of the corresponding standards. Furthermore, UV spectral characteristics of the HPLC peaks in each sample were compared with library spectra acquired from acetic and lactic acid standards. 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 quantified. Standard solutions of organic acids (lactic and acetic acid; Sigma, St. Louis, MO, USA) were prepared to establish elution times and calibration curves.

Determination of viability during refrigerated storage In separate experiments, each culture was anaerobically grown at 37°C for 48 hr with 5.0%(w/v) oligosaccharides and inulin as described previously. Controls contained no oligosaccharides or inulin. All samples were stored at 4.0 ±1.0°C for 4 weeks. A sample from each stored culture was taken at 1 week intervals to determine the viability of bifidobacteria in the fermented milk. 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 (Difco). The inoculated plates were incubated anaerobically using Gas-Paks (Becton Dickinson Co.) at 37°C for 48 hr. The colonies were counted using a Quebec colony counter (Fisher Scientific, Pittsburgh, PA, USA). Percent viability of each culture in the presence of different oligosaccharides and inulin was calculated as follows:

% viability = (CFU at each week of storage/initial CFU)× 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, CA, USA). Comparisons were made using Dunn's test for multiple comparisons. A p value <0.05 was considered statistically significant.

Results and Discussion

Growth of bifidobacteria Table 1 shows the mean doubling times for each *Bifidobacterium* spp. grown in skim milk in the presence of FOS, GOS, MOS, and inulin at concentrations 0.1, 0.5, 1.0, 3.0, and 5.0%. Mean doubling time was used as a measure of the efficacy of the various carbon sources in modulating growth rate. Growth promotion of *Bifidobacterium* spp. by oligosaccharides and inulin was observed in a dose dependent manner over a range of 0 to 5.0% as shown by decreased mean doubling times with increased concentrations of FOS, GOS, MOS, or inulin. These results indicate that all strains

Table 1. Doubling time (T_d) of *Bifidobacterium* spp. in skim milk containing oligosaccharides and inulin

Treatment	CHO level (%)	Doubling time (Td)[1)] (min)				
		B. adolescentis	*B. bifidum*	*B. breve*	*B. infantis*	*B. longum*
Control	0	278±16	258±12	315±13	286±10	363±9
Fructooligosaccharide	0.1	257±12	255±8	291±9	226±12*	324±13*
(FOS)	0.5	253±16	246±16	286±20	225±7*	304±8*
	1.0	212±13*	242±5	269±11*	195±9*	256±17*
	3.0	181±6*	236±12	208±6*	158±12*	225±12*
	5.0	177±12*	237±8*	201±22*	146±9*	192±7*
Galactooligosaccharide	0.1	253±10	246±7	303±15	263±7	345±6
(GOS)	0.5	250±4*	246±13	288±10	252±10*	330±8*
	1.0	233±2*	242±13	272±14*	245±9*	296±8*
	3.0	222±6*	249±12	233±7*	203±8*	245±7*
	5.0	208±3*	235±3*	231±11*	188±7*	236±9*
Isomaltooligosaccharide	0.1	260±13	237±8	298±20	253±8*	349±13
(MOS)	0.5	254±6	250±9	291±12	244±13*	348±8*
	1.0	238±16*	257±7	271±6*	235±10*	311±14*
	3.0	225±5*	235±9	218±15*	185±8*	231±18*
	5.0	201±10*	227±7*	209±13*	143±8*	224±10*
Inulin	0.1	268±2	242±10	303±15	260±15*	361±10
	0.5	256±5	240±8	287±8	256±13*	351±16
	1.0	244±9*	225±14*	285±11	242±11*	345±17
	3.0	219±4*	226±4*	250±19*	226±7*	337±10
	5.0	205±5*	213±9*	246±2*	209±17*	348±11

[1)]Mean doubling time (T_d) = $\ln 2/\mu$; $\mu = \ln X_2 - \ln X_1 / t_1 - t_2$. Means ± standard deviation; n=3 for all treatments.
*Indicates significantly different ($p<0.05$) from the control (comparisons are made only with the control).

grew faster in the presence of these carbohydrates compared to the control. In skim milk controls, *B. bifidum* 3418 had the lowest mean doubling time indicating this strain grew faster than other bifidobacteria tested. In contrast, *B. longum* 3128 had the slowest growth rate in skim milk. These results suggest that the growth potential of bifidobacteria in skim milk is strain specific and does not affect total yield of bifidobacteria.

Growth of *B. adolescentis* 3352 and *B. breve* 3220 increased significantly ($p<0.05$) when 1.0 to 5.0% FOS, GOS, MOS, or inulin was added. Although growth promotion of bifidobacteria by oligosaccharides and inulin was obtained in a dose-dependent manner in a concentration range of 0-5.0%, mean doubling times of these strains cultured with each compound at 3.0% were very similar. Growth of *B. infantis* 3368 was fully enhanced by all oligosaccharides tested as well as inulin, even at the lowest concentrations. Considering all the oligosaccharides tested, the order of growth-promoting activity with regard to all bifidobacteria strains except for *B. bifidum* 3418 was FOS>MOS>GOS.

In contrast to the aforementioned species, growth of *B. bifidum* 3418 was enhanced by inulin but not by FOS, GOS, or MOS. This is consistent with previous reports that *B. bifidum* cannot utilize oligosaccharides (18, 30). Oligosaccharides and inulin can be characterized by their DP values which range from 2 to 20 and from 2 to 60 or more, respectively. When inulin was added to *B. longum* 3128 cultures, the mean doubling time was similar to the control indicating that this microorganism utilized this substrate markedly slower than the other strains and may be deficient in enzymes necessary for its metabolism. Similar results have been reported by Shin *et al.* (10) in which they demonstrated that FOS and GOS were more effective than inulin. Hopkins *et al.* (32) showed that GOS and FOS, having lower DP values, were best in promoting the growth of bifidobacteria. In contrast, carbohydrates with a high DP were poor bifidobacteria substrates. The mechanism of carbohydrate uptake by bifidobacteria has not been fully clarified; however it appears likely that the substrate transport systems may be more efficient for dimeric and oligomeric carbohydrates. Dubey and Mistry (30) have reported that 0.5% FOS did not enhance the growth of bifidobacteria (*B. bifidum* ATCC 15696, *B. breve* ATCC 15700, *B. infantis* ATCC 15697, and *B. longum* ATCC 15708) in infant milk formulas containing soy or hydrolyzed casein and, on the contrary, growth of *B. breve* ATCC 15700 in either formula was inhibited by FOS after 8 hr of incubation. However, our results show that up to 5.0% FOS enhanced the growth of *B. infantis*

KTCC 3368 and *B. longum* KTCC 3128. Furthermore, 1.0% FOS enhanced the growth of *B. breve* KTCC 3220 and no inhibition was observed. These differences may relate to differences between the composition of the infant formulas and the NDM medium as well as the much higher concentration of FOS used in this study.

In accordance with growth stimulation, both acetic and lactic acid production by *Bifidobacterium* spp. was also enhanced ($p<0.05$) in the presence of 5.0% FOS, GOS, MOS, and inulin (Table 2 and 3). Production of lactic and acetic acid by *B. adolescentis* KTCC 3352 in the presence of FOS in skim milk was higher (40.4 and 95.3 mM, respectively) compared to *B. bifidum* KTCC 3418 which produced 28.1 and 55.9 mM of lactic and acetic acid, respectively. Typically, bifidobacterial fermentation results in 3 moles of acetic acid and 2 moles of lactic acid per 2 moles of glucose in an ideal synthetic medium (33). Although lactic acid production is essential in fermented dairy foods, a high concentration of acetic acid can result in an undesirable vinegar flavor in fermented dairy foods. The overall molar ratios of acetic to lactic acid in cultures of *Bifidobacterium* spp. from KTCC were higher than the theoretical molar ratio. These results are contrary to those of Shin *et al.* (10), who investigated the commercial strains of probiotic *Bifidobacterium* spp. that are used in the manufacture of dairy products.

Since the growth of bifidobacteria was significantly affected by the type of oligosaccharides and inulin, the initial viable cell counts varied prior to refrigerated storage (immediately after 24 hr cultivation with or without 5.0% oligosaccharides and inulin). Therefore, to compare effects on viability, colony forming units CFU/mL were determined and used to calculate percent viability (Table 4). The initial viability corresponded to 100% for all strains. *Bifidobacterium* strains in skim milk controls exhibited a significant loss in viability within the first week of refrigerated storage. Only 26.7 to 37.6% of viable bifidobacteria remained. All strains exhibited decreases in viable populations after 4 weeks to less than 5.0% of the initial counts. *B. infantis* KTCC 3368 exhibited the highest viability (4.4±1.3%), whereas the viability of *B. longum* KTCC 3128 was lowest (0.8±0.1%) in fermented milk after the 4 week of storage at 7°C. Except for *B. bifidum* KTCC 3418, the viability of bifidobacteria cultured with 5.0% FOS and, to a lesser extent, MOS was higher ($p<0.05$) than the control after 4 weeks of storage. The viability of *B. adolescentis* KTCC 3352 was enhanced most by FOS. When bifidobacteria were cultured with 5.0% GOS, the viabilities at different storage times were similar to that of the control except at 3 and 4 weeks of storage for *B. adolescentis* KTCC 3352 and 3 weeks of storage for *B. longum* KTCC 3128. The viability of bifidobacteria cultured with 5.0% inulin was similar to the control except at 4 weeks for *B. bifidum* KTCC 3418 and at 1 to 2 weeks for *B. adolescentis* KTCC 3352 and *B. infantis* KTCC 3368. These results indicate that FOS and MOS are most effective in enhancing viability of bifidobacteria, whereas GOS and inulin are not effective.

The loss of viability of bifidobacteria is more marked in fermented milk than in unfermented milk (34, 35). Acid sensitivity was probably a factor in decreased cell viability. Thus, low temperature storage, suspension of metabolic activity, tolerance to pH change, and low redox potential may have enhanced the survivability of organisms in milk. Our results differ from previous reports showing that the addition of FOS had no effect on the viability of

Table 2. Lactic acid production by *Bifidobacterium* spp. in skim milk containing 5% oligosaccharides and inulin[1,2)]

Treatment	Lactic Acid (mM)				
	Control	FOS	GOS	MOS	Inulin
B. adolescentis (KTCC 3352)	5.6±3.8[a]	40.4±4.5[b]	38.6±2.9[b]	32.6±3.6[b]	31.8±2.3[b]
B. bifidum (KTCC 3418)	4.8±2.7[a]	28.1±2.5[b]	19.3±2.1[b]	17.5±2.0[b]	15.8±1.4[b]
B. breve (KTCC 3220)	6.2±5.8[a]	30.9±3.1[b]	33.5±3.3[b]	27.2±2.8[b]	25.4±2.4[b]
B. infantis (KTCC 3368)	5.2±5.3[a]	35.0±4.1[b]	41.6±3.3[b]	29.8±2.8[b]	31.5±3.1[b]
B. longum (KTCC 3128)	4.9±1.3[a]	36.4±3.5[b]	31.0±2.6[b]	28.2±2.2[b]	26.7±1.9[b]

[1)]Means with different superscripts are significantly different ($p<0.05$). Comparisons are made only within the same row.
[2)]Means±standard deviations; n=3 for all treatments.

Table 3. Acetic acid production by *Bifidobacterium* spp. in skim milk containing 5% oligosaccharides and inulin[1,2)]

Treatment	Acetic Acid (mM)				
	Control	FOS	GOS	MOS	Inulin
B. adolescentis (KTCC 3352)	12.3±1.2[a]	95.3±10.1[b]	93.9±10.2[b]	83.9±9.8[b]	85.8±12.9[b]
B. bifidum (KTCC 3418)	13.9±1.0[a]	55.9±7.6[b]	45.9±8.4[b]	48.3±6.7[b]	44.6±7.4[b]
B. breve (KTCC 3220)	16.4±1.2[a]	78.2±8.5[b]	81.3±9.0[b]	69.9±11.6[b]	67.7±10.5[b]
B. infantis (KTCC 3368)	18.4±1.2[a]	88.4±14.1[b]	79.1±9.5[b]	74±8.9[b]	71.6±9.8[b]
B. longum (KTCC 3128)	15.5±1.1[a]	82.1±7.9[b]	72.4±6.8[b]	66.7±7.1[b]	71.4±7.5[b]

[1)]Means with different superscripts are significantly different ($p<0.05$). Comparisons are made only within the same row.
[2)]Means±standard deviations; n=3 for all treatments.

Table 4. Viability of *Bifidobacterium* spp. grown in skim milk containing 5% oligosaccharides and inulin during 4 week of refrigerated storage at 4°C

Treatment	Week	% Viability[1)]				
		Control	FOS	GOS	MOS	Inulin
B. adolescentis	1	26.7±3.3^{a}	46.0±3.9^{b}	30.0±1.9^{a}	42.4±9.4^{b}	41.2±4.7^{b}
KTCC 3352	2	10.5±1.0^{b}	12.9±0.3^{c}	9.1±0.7^{a,b}	8.9±1.1^{a,b}	8.7±1.2^{a}
	3	3.1±0.9^{a}	8.5±0.5^{d}	4.8±0.4^{b,c}	5.5±0.4c	3.7±0.9^{a,b}
	4	1.1±0.4^{a}	3.7±0.7^{c}	2.1±0.3^{b}	1.9±0.4b	1.6±0.2^{a,b}
B. bifidum	1	37.6±2.0^{a}	34.4±12.3^{a}	40.5±9.6^{a}	33.6±12.0^{a}	39.3±14.3^{a}
KTCC 3418	2	13.1±1.0^{a}	13.1±2.6^{a}	16.4±1.4^{a}	13.7±4.8^{a}	15.4±0.9^{a}
	3	11.6±1.4^{c}	8.9±0.9^{a,b}	7.4±1.8^{a}	9.7±0.6^{b,c}	8.1±1.3^{a,b}
	4	2.4±0.2^{a}	2.2±0.7^{a}	2.5±0.3^{a}	2.1±0.4^{a}	3.2±0.2^{b}
B. breve	1	30.1±9.3^{a}	51.2±11.0^{b}	36.5±5.3^{a}	47.5±4.0^{a,b}	33.9±3.4^{a}
KTCC 3220	2	13.6±0.3^{a}	19.4±4.2^{b}	15.5±0.4^{a,b}	15.2±2.5^{a}	12.6±0.4^{a}
	3	4.3±2.1^{a}	7.8±0.8^{b}	6.4±0.9^{a,b}	6.6±0.7^{a,b}	5.0±0.9^{a}
	4	2.7±0.6^{a}	4.5±0.5^{b}	3.1±0.5^{a,b}	4.5±1.3^{b}	2.8±0.7^{a}
B. infantis	1	29.5±6.2^{a}	38.2±5.8^{a,b}	30.0±6.1^{a}	57.9±19.3^{b}	89.1±20.0^{c}
KTCC 3368	2	15.7±5.7^{a}	19.4±2.6^{a,b}	20.5±1.1^{a,b}	17.9±3.5^{a}	24.4±1.2^{b}
	3	10.7±5.1^{a}	15.3±1.7^{a}	12.0±2.0^{a}	12.1±5.7^{a}	13.2±0.4^{a}
	4	4.4±1.3^{a}	8.5±1.5^{b}	6.1±0.7^{a}	8.4±0.5^{b}	4.9±0.9^{a}
B. longum	1	36.9±5.9^{a}	44.0±0.9^{a}	41.9±15.4^{a}	46.7±3.7^{a}	37.0±7.1^{a}
KTCC 3128	2	10.0±1.6^{a}	14.8±1.4^{b}	13.8±4.4^{a,b}	10.5±0.5^{a,b}	11.6±1.3^{a,b}
	3	2.3±0.5^{a}	7.0±0.9^{c}	4.5±0.3^{b}	4.4±0.1^{b}	2.0±0.5^{a}
	4	0.8±0.1^{a}	2.6±0.1^{c}	1.3±0.6^{a,b}	1.9±0.8^{b,c}	1.0±0.2^{a}

[1)]% viability = (CFU after 4 week storage/initial CFU) × 100. Means with different letters are significantly different ($p<0.05$). Comparisons are made only within the same row. Means±standard deviation; n=3 for all treatments.

bifidobacteria in ice cream (36) or Edam cheese (37). In thesestudies, bifidobacteria and FOS were mixed with ice cream or Edam cheese and stored at -17°C or at 7°C, respectively. In our study, bifidobacteria were allowed to grow and utilize FOS for 24 hr of fermentation before starting refrigerated storage, thus the presence of FOS may have contributed to the available nutrients and caused greater viability. Enhanced viability of commercial strains of *Bifidobacterium* spp. (Bf-1 and Bf-6) during 4 weeks of refrigerated storage has been reported by Shin *et al.* (10) when those organisms were grown in the presence of 5.0% FOS and GOS. They reported 67 and 52% retention of viability of Bf-1 with FOS and GOS, respectively, whereas the retention of viability of Bf-6 was 44 and 39% in the presence of FOS and GOS, respectively. Further studies are needed to elucidate the relation of oligosaccharides to the viability of bifidobacteria and the mechanism of carbohydrate utilization by bifidobacteria.

The degree of enhancement of growth, activity, and viability of the five strains of bifidobacteria in milk were dependent on the carbon source as well as the bifidobacteria strain. FOS provided the best growth-promoting activity for all the bifidobacteria tested except for *B. bifidum* KTCC 3418. Bifidobacteria cultures grown with FOS also exhibited the highest viability after 4 weeks of refrigerated storage compared to the control. Although our results indicate that the bifidobacteria strain and the type of carbon source are significant in the incorporation of these organisms into fermented dairy foods, additional information is needed regarding commercial strains of bifidobacteria that are used as dietary adjuncts to optimize their growth and viability in commercial dairy products.

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