Effect of Bifidogenic Factors on Growth Characteristics of Bifidobacteria in Infant Formulas[1]

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ABSTRACT

A bifidogenic factor, lactulose or fructooligosaccharides, was added (0.5%) into infant formula based on soy or infant formula with hydrolyzed casein. The infant formulas were then inoculated (2.5%) with *Bifidobacterium bifidum* (ATCC 15696), *Bifidobacterium breve* (ATCC 15700), *Bifidobacterium infantis* (ATCC 15697), or *Bifidobacterium longum* (ATCC 15708) or their mixture (mixed culture) and incubated at 37°C for 24 h. Lactulose did not influence maximal counts or generation times in either formula for any species except *B. infantis*, which had lower counts. Trends of developed acidity and pH of the mixed culture in the infant formulas with or without lactulose were similar to those for *B. breve*. Maximal counts and generation times remained unchanged with or without fructooligosaccharides for all species and the mixed culture, except for *B. bifidum* in the formula based on soy, for which maximal counts did not occur. Growth in either formula was inhibited for *B. infantis* with lactulose and *B. breve* with fructooligosaccharides past 8 h of inoculation.
(**Key words**: bifidobacteria, infant milk formula, lactulose, fructooligosaccharides)

Abbreviation key: **CH** = infant formula with hydrolyzed casein, **CHF** = CH with 0.5% FOS, **CHL** = CH with 0.5% lactulose, **DA** = developed acidity, **FOS** = fructooligosaccharides, **SB** = infant formula based on soy, **SBF** = SB with 0.5% FOS, **SBL** = SB with 0.5% lactulose.

INTRODUCTION

Many researchers (10, 11, 16, 26) have described roles of bifidobacteria as therapeutic and prophylactic agents for human and animal health. Dairy products containing bifidobacteria have potential benefits for infants and adults that are generally related to inhibition of pathogens, maintenance and restoration of normal intestinal flora (2, 3, 5, 26) increased immune response (3, 7, 19, 34, 36), and detoxification of metabolites by lowering blood ammonia, phenol, and urinary indican in the gastrointestinal tract (3, 6).

Bifidobacteria grow better in human milk than in bovine milk in part because of the lower protein and buffering capacity of the former (5) and because of the presence of lactoferrin and transferrin (27). Bifidobacteria are nutritionally fastidious (24); the difficulty experienced in maintaining their viability over long periods led to the search for growth promoters. Well-studied and widely used complex carbohydrates, lactulose and fructooligosaccharides (**FOS**), are indigestible by humans but can be metabolized by bifidobacteria in the lower gut (1, 8, 12, 14, 15, 17, 29, 30). Effects of those growth parameters on fecal pH and bifidobacterial counts in infants have been studied (14, 18, 22, 32).

The FOS are naturally occurring, complex carbohydrates, the consumption of which increases the population of bifidobacteria in the colon (33). In Japan, FOS are considered to be food, not food ingredients, and are found in more than 500 food products (29). Products of FOS, known as neosugars, are produced by the action of a fungal (*Aspergillus niger*) β-fructofuranosidase on sucrose (8) and have been evaluated by various researchers (12, 31). Growth promotion of casein hydrolyzates and other protein-based materials has also been tested (13, 23, 25).

In a previous study (8), growth of four species of bifidobacteria in infant formulas based on soy (**SB**) or with hydrolyzed casein (**CH**) or milk-based, was examined. Growth was generally lower in SB and CH than in milk-based formula and nonfat milk. The objective of this study was to evaluate the effect of lactulose and FOS on the growth characteristics in vitro of four different species of bifidobacteria and a mixture of these species in SB and CH.

MATERIALS AND METHODS

Infant Formulas and Cultures

Commercial SB and CH, selected from at least three different lots, were used. These infant formulas

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were presterilized and ready to use. Lyophilized *Bifidobacterium bifidum* 15696, *Bifidobacterium breve* 15700, *Bifidobacterium infantis* 15697, and *Bifidobacterium longum* 15708 were from American Type Culture Collection (Rockville, MD). Cultures were rehydrated in modified lactobacilli MRS medium (Difco Laboratories, Detroit, MI) containing 0.05% L-cysteine·HCl (Sigma Chemical Co., St. Louis, MO) (35) and incubated anaerobically for 24 h at 37°C without agitation using the GasPak® culture system (Becton Dickinson Microbiology Systems, Cockeysville, MD). A mixed culture was prepared by combining individual species of bifidobacteria after they were grown for 12 to 16 h at 37°C in MRS broth under anaerobic environment.

Analytical Methods

Bacterial growth. Samples were collected aseptically at 0 and every 4 h postinoculation until 24 h. Bifidobacteria were enumerated (35) using an incubation temperature of 37°C for 48 to 72 h under anaerobic conditions as described. Generation times were determined from the log phase of growth (20).

Acid production. Titratable acidity was measured by titration with 0.1*N* NaOH to the phenolphthalein end point (pH 8.3) (4) and reported as a percentage of developed acidity (**DA**), the difference between total and original titratable acidity. A Corning ion analyzer 150, equipped with a temperature compensation probe (Corning Medical and Scientific, Medfield, MA), was used to measure pH at 25°C.

Lactic and acetic acid production. Acetic and lactic acids were determined as previously described (8). Samples were analyzed on a Spectra Physics HPLC system (Spectra Physics Inc., San Jose, CA) using an SP 8430 refractive index detector, SP 8875 autosampler, Chromjet SP 4400 integrator, and HPX-87H column (Bio-Rad, Hercules, CA) operated at 65°C and a flow rate of 6 ml/min. The mobile phase used was 0.1*N* H_2SO_4. The HPLC system was calibrated using 0.05-, 0.5-, and 5-g/L standards for lactic and acetic acids.

Bifidogenic Factors

Lactulose (Sigma Chemical Co.) and FOS (Golden Technologies Co., Inc., Westminster, CO) were used in SB and CH at the rate of 0.5% as follows: SB with lactulose (**SBL**) or with FOS (**SBF**) and CH with lactulose (**CHL**) or with FOS (**CHF**). The SB and CH without bifidogenic factors were used as controls. Each of the formulas, with or without bifidogenic factors, was inoculated at 2.5% with each stock culture and incubated in a water bath at 37°C for 24 h.

Statistical Analysis

Data were analyzed using PC-SAS® (28). Most data followed a multivariate design with repeated measures in which time was the dependent variable. The mean maximal bacterial counts (transformed to $\log_{10}$), generation time, pH, DA, acetic and lactic acids, and molar ratio of acetic to lactic acid at maximal bacterial counts were analyzed using least squares means. Least squares means were separated using the Student's *t* test. Experiments were replicated three times.

Prior to statistical analysis, data that were not normally distributed were transformed as follows. Counts, generation times, and developed acidity data were transformed to logs, and data for acetic and lactic acid were transformed to square roots. Data presented in the paper represent untransformed means.

RESULTS

Growth

Lactulose. The effect of lactulose on the growth characteristics of bifidobacteria was minimal. Mean maximal counts (Table 1) and generation times (Table 2) of all species for three replicates in SBL and CHL were similar ($P > 0.05$) to those of their corresponding controls, SB and CH, respectively. The only exception was *B. infantis* for which counts in CHL were lower ($P < 0.05$) than for the control, CH (Table 1). Maximal counts and generation times for the mixed culture in SBL and CHL were similar to those of the corresponding controls ($P > 0.05$) and mimicked those of *B. breve*. Log phase of growth for the four species and the mixed culture in SBL and CHL were observed during the first 4 to 8 h postinoculation.

FOS. Log phase for *B. bifidum* did not occur in SBF and SB. Therefore, no data were obtained at maximal counts for this species in SB and SBF. The mean maximal counts for three replicates of all species in SBF and CHF were similar ($P > 0.05$) to those of their corresponding controls, SB and CH, respectively. Counts for *B. breve* in SBF, CHF, and the controls were higher ($P < 0.05$) than those of the other three species and the mixed culture (Table 1).

Generation times in SBF ranged from 70 (*B. infantis*) to 112 min (mixed culture) and in CHF from 112 (*B. infantis*) to 347 min (mixed culture) (Table 2).

TABLE 1. Effect of lactulose and fructooligosaccharides on maximal counts of bifidobacteria in infant formulas.[1]

Bifidobacterium species	Formula			
	SB	SBL	CH	CHL
	(log_{10} cfu/ml)			
Lactulose				
B. bifidum	$6.96^{c,B}$	$6.90^{d,B}$	$7.23^{c,A}$	$7.23^{d,A}$
B. breve	$9.34^{a,A}$	$9.28^{a,A}$	$8.50^{a,B}$	$8.52^{a,B}$
B. infantis	$8.59^{b,A}$	$8.54^{c,A}$	$7.88^{b,B}$	$7.53^{c,C}$
B. longum	$8.73^{b,A}$	$8.76^{b,A}$	$7.81^{b,B}$	$7.86^{b,B}$
Mixed culture[2]	$9.15^{a,A}$	$9.15^{a,A}$	$8.54^{a,B}$	$8.66^{a,B}$
Fructooligosaccharides	SB	SBF	CH	CHF
B. bifidum	NM[3]	NM	$7.25^{d,A}$	$7.23^{d,A}$
B. breve	$9.28^{a,A}$	$9.23^{a,A}$	$8.64^{a,B}$	$8.43^{a,B}$
B. infantis	$8.61^{c,A}$	$8.76^{b,A}$	$7.83^{b,B}$	$7.73^{c,B}$
B. longum	$8.87^{b,A}$	$8.86^{b,A}$	$7.72^{b,B}$	$7.75^{c,B}$
Mixed culture	$8.76^{bc,A}$	$8.80^{b,A}$	$7.98^{c,B}$	$7.87^{b,B}$

^{a,b,c,d}Means (n = 3) in columns with no common superscripts within bifidogenic factor type differ ($P < 0.05$).

^{A,B,C}Means (n = 3) in rows with no common superscripts differ ($P < 0.05$).

[1]SB = Infant formula based on soy, SBL = SB with 0.5% lactulose, CH = infant formula with hydrolyzed casein, CHL = CH with 0.5% lactulose, SBF = SB with 0.5% fructooligosaccharide, and CHF = CH with 0.5% fructooligosaccharide.

[2]Mixed culture = Mixture of *B. bifidum*, *B. breve*, *B. infantis*, and *B. longum*.

[3]Not measurable because of absence of log phase.

Generation times of all of the species in SBF were similar ($P > 0.05$) to those in SB. Generation times of all of the species except *B. longum* in CHF were similar ($P > 0.05$) to those in CH. The mean log phase of growth for all of the species in the infant formulas occurred during the first 4 to 10 h of fermentation.

Acid Production

Lactulose. At maximal counts in SBL, the mean DA and pH by all four species were different ($P < 0.05$) from one another and similar ($P > 0.05$) to SB, except for *B. infantis*, which produced more acid in SBL (Table 3). In CHL, the mean DA and pH for all four species were different ($P < 0.05$) from one another and similar ($P > 0.05$) to CH except for *B. breve* and *B. infantis*. There were no differences ($P > 0.05$) in the DA and pH of the mixed culture between SB and SBL or between CH and CHL. Trends in the DA and pH of mixed culture and *B. breve* were similar ($P > 0.05$) in both the treatments and the control.

FOS. The mean DA of all species at maximal counts in SBF were similar to one another and not different ($P > 0.05$) from that of the control, SB (Table 3). In SBF, pH differed ($P < 0.05$) between *B. breve* and *B. infantis*, but in SB pH were similar ($P > 0.05$) for all species. There were no differences in the pH between SBF and the control for any species. In CHF and CH, the mean DA and pH of all four species were significantly different ($P < 0.05$) from one another, but DA and pH of the four species and mixed culture were similar ($P > 0.05$) to those of their controls.

Production of Lactic and Acetic Acids

Lactulose. The acetic and lactic acids at maximal counts in SBL ranged from 4.2 and 4.4 m*M* (*B. bifidum*) to 26.9 and 22.6 m*M* (*B. infantis*), giving molar ratios for acetic to lactic acid of 0.95 and 1.19, respectively (Table 4). In CHL, the range was 4.8 and 7.9 m*M* (*B. bifidum*) to 19.6 and 19.4 m*M* (*B. infantis*), and the corresponding ratios ranged from 0.61 to 1.01, respectively. Production of these acids by *B. infantis* in SBL and CHL was higher ($P < 0.05$) than those of their corresponding controls. For mixed culture, production of acetic and lactic acids in SBL and CHL was similar ($P > 0.05$) to that of their respective controls and similar to those of *B. breve*. Ratios of acetic to lactic acids in SBL and CHL for *B. infantis* were lower ($P < 0.05$) than those of their controls. Ratios for mixed culture in SBL and CHL were 1.12 and 1.10, which were similar ($P > 0.05$) to their respective controls.

TABLE 2. Effect of lactulose and fructooligosaccharides on generaton times of bifidobacteria during fermentation in infant formulas.[1]

Bifidobacterium species	Formula			
	SB	SBL	CH	CHL
	(min)			
Lactulose				
B. bifidum	$296^{a,A}$	$201^{a,AB}$	$158^{bc,BC}$	$139^{bc,C}$
B. breve	$80^{bc,B}$	$86^{bc,B}$	$237^{a,A}$	$218^{a,A}$
B. infantis	$66^{c,A}$	$59^{c,A}$	$87^{c,A}$	$91^{c,A}$
B. longum	$105^{bc,B}$	$106^{b,B}$	$333^{a,A}$	$256^{a,A}$
Mixed culture[2]	$112^{b,B}$	$108^{b,B}$	$218^{ab,A}$	$211^{ab,A}$
Fructooligosaccharides	SB	SBF	CH	CHF
B. bifidum	NM[3]	NM	$175^{bc,A}$	$132^{c,A}$
B. breve	$99^{a,B}$	$108^{a,B}$	$177^{b,A}$	$234^{b,A}$
B. infantis	$72^{b,B}$	$70^{b,B}$	$130^{c,A}$	$112^{c,A}$
B. longum	$97^{ab,C}$	$101^{a,C}$	$324^{a,A}$	$226^{b,B}$
Mixed culture	$117^{a,B}$	$112^{a,B}$	$284^{a,A}$	$347^{a,A}$

[a,b,c,d]Means (n = 3) in columns with no common superscripts within bifidogenic factor type differ ($P < 0.05$).

[A,B,C]Means (n = 3) in rows with no common superscripts differ ($P < 0.05$).

[1]SB = Infant formula based on soy, SBL = SB with 0.5% lactulose, CH = infant formula with hydrolyzed casein, CHL = CH with 0.5% lactulose, SBF = SB with 0.5% fructooligosaccharide, and CHF = CH with 0.5% fructooligosaccharide.

[2]Mixed culture = Mixture of *B. bifidum*, *B. breve*, *B. infantis*, and *B. longum*.

[3]Not measurable because of absence of log phase.

FOS. Acetic acid at maximal counts in SBF ranged from 19.3 (mixed culture) to 21.0 mmol (*B. infantis*) and lactic acid ranged from 14.9 (*B. breve*) to 18.8 mmol (*B. longum*) (Table 4). There were no differences ($P > 0.05$) in the production of acetic and lactic acids by any species or mixed culture in SBF. In CHF, the acetic and lactic acids ranged from 5.0 and 2.3 (*B. bifidum*) to 15.7 and 11.6 m*M* (*B. breve*), respectively. The acetic acid (12.1 m*M*) and lactic acid (13.8 m*M*) of mixed culture were similar ($P > 0.05$) to those of *B. breve*. There were no differences ($P > 0.05$) in the concentrations of these acids produced by any of the four species or mixed culture between CHF and CH. The mean ratios of acetic to lactic acid in SBF for all species were <1.5 and were similar ($P > 0.05$) to one another and to those in SB. In CHF, the ratios were >1.5 for *B. bifidum* and *B. infantis* but <1.5 for *B. longum*, *B. breve*, and mixed culture and were similar ($P > 0.05$) to those for CH for all species.

DISCUSSION

Maximal bacterial counts and generation times in SBL and SB were similar to one another for all species and similar to the mixed culture but varied ($P < 0.05$) among species, once again suggesting specificity of species in the formulas and also indicating that lactulose was ineffective in enhancing growth. Maximal counts for the mixed culture were similar to those for *B. breve* in SBL and SB. Trends for maximal counts in CHL and CH were similar to those in SBL and SB for all species and mixed culture except *B. infantis*. Some lactococci preferred utilization of glucose or lactose over utilization of galactose or other sugars (21). Some bifidobacteria might also prefer metabolizing some sugars over lactulose. Also, counts in SBL and CHL might possibly increase later during fermentation when the inherent sugars are exhausted. Compared with counts for the controls, counts for *B. infantis* in SBL and in CHL continued to decline gradually after 8 h of incubation (Figure 1a). This type of decline was not observed with the other species.

Maximal counts did not occur for *B. bifidum* in SB with or without FOS, possibly because of inhibition of this species in SB, which would also confirm results of our earlier study (8). Maximal counts of all species and the mixed culture in SBF and CHF were similar to those in SB and CH, respectively, indicating no response to FOS. Although maximal counts of *B. breve* in SBF and CHF were similar ($P > 0.05$) to those in their controls (Table 1), counts for this species in the formulas with FOS were lower ($P < 0.05$) than in the controls past 8 h of incubation, indicating inhibition of growth in the presence of FOS (Figure 2a). This phenomenon was similar to that observed with lactulose (Figure 1a).

TABLE 3. Effect of lactulose and fructooligosaccharides on developed acidity (DA)[1] and pH at maximal bacterial counts for bifidobacteria in infant formulas.[2]

Bifidobacterium species	Formula							
	SB		SBL		CH		CHL	
	DA	pH	DA	pH	DA	pH	DA	pH
	(%)		(%)		(%)		(%)	
Lactulose								
B. bifidum	0.02^{c,A}	6.20^{a,A}	0.02^{d,A}	6.20^{a,A}	0.02^{d,A}	5.31^{a,B}	0.05^{d,C}	5.18^{a,C}
B. breve	0.36^{a,AB}	4.66^{c,AB}	0.41^{b,A}	4.62^{c,AB}	0.26^{b,C}	4.71^{cd,A}	0.32^{b,B}	4.57^{c,B}
B. infantis	0.24^{b,C}	4.94^{b,A}	0.51^{a,A}	4.47^{d,B}	0.13^{c,D}	4.97^{b,A}	0.39^{a,B}	4.51^{c,B}
B. longum	0.28^{b,A}	4.92^{b,A}	0.29^{c,A}	4.92^{b,A}	0.28^{b,A}	4.80^{c,B}	0.24^{c,A}	4.79^{b,B}
Mixed culture[3]	0.35^{a,AB}	4.72^{c,A}	0.38^{b,A}	4.69^{c,A}	0.30^{a,B}	4.64^{d,AB}	0.34^{b,AB}	4.58^{c,B}
Fructooligosaccharides	SB		SBF		CH		CHF	
	DA	pH	DA	pH	DA	pH	DA	pH
B. bifidum	NM[4]	NM	NM	NM	0.03^{d,A}	5.22^{a,A}	0.03^{d,A}	5.22^{a,A}
B. breve	0.34^{a,A}	4.77^{a,A}	0.34^{a,A}	4.80^{a,A}	0.29^{a,A}	4.72^{c,A}	0.29^{a,A}	4.74^{c,A}
B. infantis	0.34^{a,A}	4.72^{a,B}	0.37^{a,A}	4.68^{c,B}	0.16^{b,B}	4.93^{d,A}	0.16^{b,B}	4.92^{d,A}
B. longum	0.34^{a,A}	4.72^{a,B}	0.35^{a,A}	4.71^{bc,B}	0.09^{c,B}	5.09^{b,A}	0.09^{c,B}	5.10^{b,A}
Mixed culture	0.32^{a,A}	4.79^{a,A}	0.34^{a,A}	4.78^{ab,A}	0.21^{b,B}	4.83^{e,A}	0.21^{b,B}	4.83^{c,A}

^{a,b,c,d}Means (n = 3) in columns with no common superscripts within bifidogenic factor type differ ($P < 0.05$).

^{A,B,C}Means (n = 3) in rows with no common superscripts differ ($P < 0.05$).

[1]Total titratable acidity minus initial titratable acidity.

[2]SB = Infant formula based on soy, SBL = SB with 0.5% lactulose, CH = infant formula with hydrolyzed casein, and CHL = CH with 0.5% lactulose, SBF = SB with 0.5% fructooligosaccharide, and CHF = CH with 0.5% fructooligosaccharide.

[3]Mixed culture = Mixture of *B. bifidum*, *B. breve*, *B. infantis*, and *B. longum*.

[4]Not measurable because of absence of log phase.

TABLE 4. Effect of lactulose and fructooligosaccharides on concentrations of and ratios[1] of acetic (AA) to lactic (LA) acids produced at maximal counts for bifidobacteria during growth in infant formulas.[2]

Bifidobacterium species	Formula											
	SB			SBL			CH			CHL		
	AA	LA	Ratio	AA	LA	Ratio	AA	LA	Ratio	AA	LA	Ratio
	— (m*M*) —			— (m*M*) —			— (m*M*) —			— (m*M*) —		
Lactulose												
B. bifidum	4.0^{c,A}	4.1^{d,B}	0.98^{b,A}	4.2^{d,A}	4.4^{c,B}	0.95^{b,A}	5.0^{d,A}	7.6^{c,A}	0.66^{c,A}	4.8^{c,A}	7.9^{c,A}	0.61^{b,A}
B. breve	18.2^{ab,B}	18.5^{a,A}	0.99^{b,A}	24.7^{ab,A}	20.9^{a,A}	1.18^{a,,A}	13.9^{bc,C}	11.9^{b,C}	1.17^{b,A}	16.7^{a,BC}	15.2^{b,B}	1.09^{a,A}
B. infantis	15.4^{b,C}	8.2^{c,C}	1.87^{a,B}	26.9^{a,A}	22.6^{a,A}	1.19^{a,C}	11.6^{c,D}	3.6^{d,D}	3.21^{a,A}	19.6^{a,B}	19.4^{a,B}	1.01^{a,C}
B. longum	15.8^{b,AB}	14.5^{b,B}	1.09^{b,A}	17.1^{c,A}	15.8^{b,AB}	1.09^{a,A}	14.8^{ab,AB}	17.4^{a,A}	0.85^{c,A}	13.1^{b,B}	14.5^{b,B}	0.90^{ab,A}
Mixed culture[3]	20.3^{a,A}	18.0^{a,A}	1.12^{ba,A}	21.3^{b,A}	16.8^{b,A}	1.27^{a,A}	17.5^{a,A}	14.0^{b,B}	1.26^{b,A}	19.1^{a,A}	17.4^{ab,A}	1.10^{a,A}
Fructooligosaccharides	SB			SBF			CH			CHF		
	AA	LA	Ratio	AA	LA	Ratio	AA	LA	Ratio	AA	LA	Ratio
B. bifidum	NM[4]	NM	NM	NM	NM	NM	5.0^{c,A}	2.1^{c,A}	2.36^{b,A}	5.0^{d,A}	2.3^{c,A}	2.20^{b,A}
B. breve	20.1^{a,A}	17.5^{ab,A}	1.15^{b,A}	20.3^{a,A}	14.9^{a,AB}	1.36^{a,A}	16.9^{a,AB}	12.4^{a,B}	1.36^{cd,A}	15.7^{a,B}	11.6^{a,B}	1.35^{c,A}
B. infantis	23.4^{a,A}	14.4^{b,A}	1.62^{a,B}	21.0^{a,A}	16.7^{a,A}	1.26^{a,B}	10.6^{b,B}	3.8^{bc,B}	2.81^{a,A}	10.3^{bc,B}	3.8^{bc,B}	2.69^{a,A}
B. longum	20.0^{a,A}	18.9^{a,A}	1.06^{b,A}	20.4^{a,A}	18.8^{a,A}	1.09^{a,AB}	7.4^{c,B}	5.2^{b,B}	1.43^{c,B}	7.3^{cd,B}	5.3^{b,B}	1.38^{c,AB}
Mixed culture	15.5^{b,AB}	18.1^{ab,A}	0.86^{b,A}	19.3^{a,B}	17.4^{a,A}	1.11^{a,A}	12.8^{b,A}	13.6^{a,B}	0.94^{d,A}	12.1^{ab,A}	13.8^{a,B}	0.88^{d,A}

^{a,b,c,d}Means (n = 3) in columns with no common superscripts within bifidogenic factor type differ ($P < 0.05$).

^{A,B,C,D}Means (n = 3) in rows with no common superscripts differ ($P < 0.05$).

[1]Ratio of means of AA and LA.

[2]SB = Infant formula based on soy, SBL = SB with 0.5% lactulose, CH = infant formula with hydrolyzed casein, and CHL = CH with 0.5% lactulose, SBF = SB with 0.5% fructooligosaccharide, and CHF = CH with 0.5% fructooligosaccharide.

[3]Mixed culture = Mixture of *B. bifidum*, *B. breve*, *B. infantis*, and *B. longum*.

[4]Not measurable because of absence of log phase.

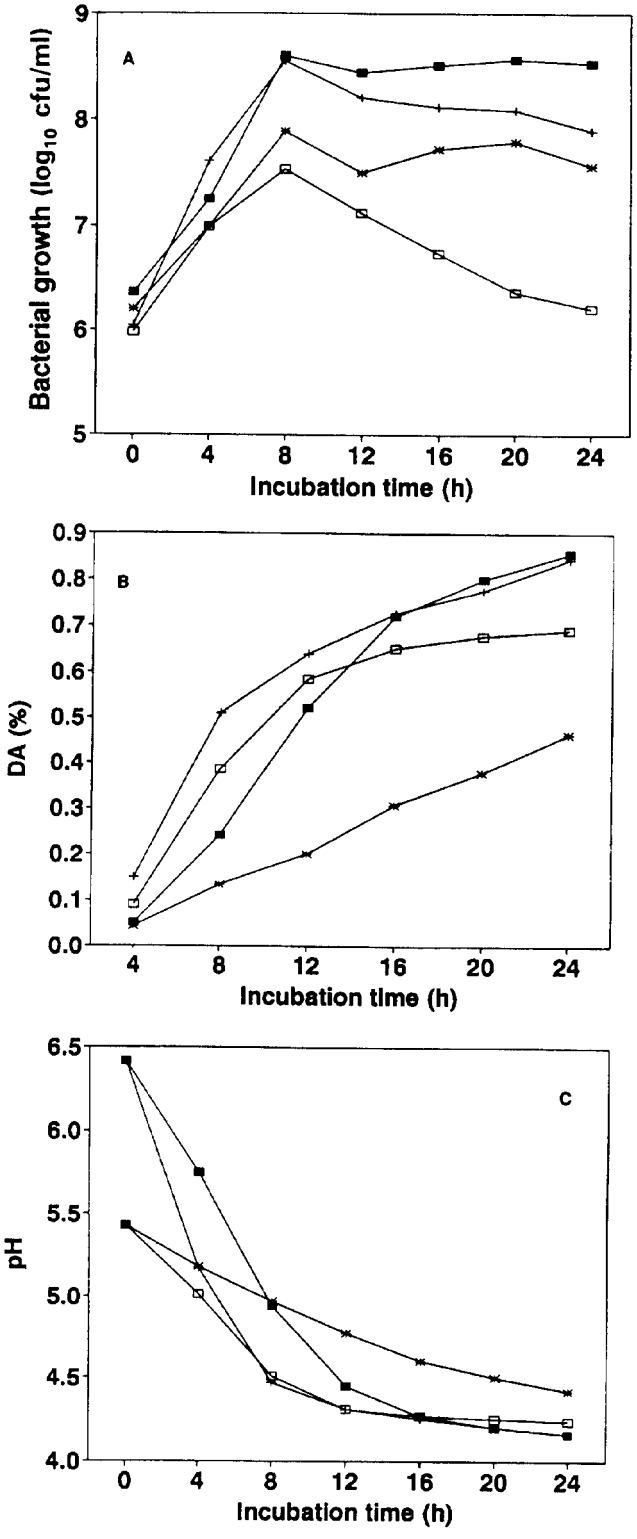

Figure 1. Effect of lactulose on counts (A), developed acidity (DA; B), and pH (C) of *Bifidobacterium infantis* during 24 h of incubation in infant formulas based on soy (■), soy with 0.5% lactulose (+), hydrolyzed casein (*), or hydrolyzed casein with 0.5% lactulose (□).

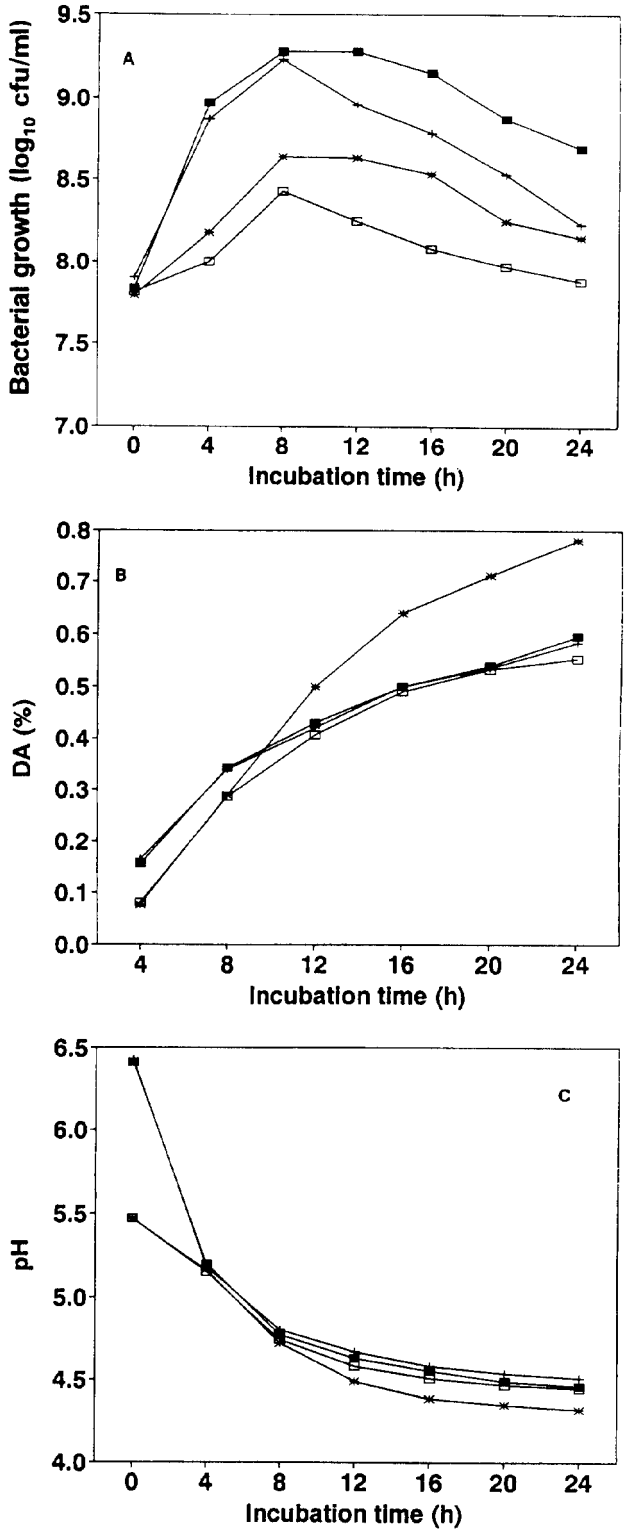

Figure 2. Effect of fructooligosaccharides on counts (A), developed acidity (DA; B), and pH (C) of *Bifidobacterium breve* during 24 h of incubation in infant formula based on soy (■), soy with 0.5% lactulose (+), hydrolyzed casein (*), or hydrolyzed casein with 0.5% lactulose (□).

The DA and pH in SBL and SB were similar ($P > 0.05$) for all species and in the mixed culture except for *B. infantis* (Table 3). Interestingly, counts in CHL for *B. infantis* were lower ($P < 0.05$) than those in CH (Figure 1a), but acid production was higher (Figure 1b), suggesting that higher ($P < 0.05$) acid production and lower pH ($P < 0.05$) (Figure 1c) for *B. infantis* in the presence of lactulose might have inhibited survival of *B. infantis* during the stationary phase of growth. Also, higher DA and lower pH in SBL and CHL for *B. infantis* indicated a higher degree of uncoupling of acid production from growth. The mixed culture was not influenced by lactulose.

Growth or acid production of either species in either formula was not enhanced by FOS. For *B. breve*, acid production (Figure 2b) and change in pH (Figure 2c) in CHF were lower ($P < 0.05$) than those in the control after 8 h postinoculation, which was in agreement with the lower ($P < 0.05$) *B. breve* counts in CHF than in the control (Figure 2a). The mixed culture of bifidobacteria remained unaffected by FOS in SB and CH.

The ratio of acetic to lactic acids at maximal counts was not influenced by lactulose and was <1.5 for all species and the mixed culture, except for *B. infantis* for which ratios were <1.5 in SBL and CHL but >1.5 in their corresponding controls (Table 4). This result was caused by higher ($P < 0.05$) production of acetic and lactic acids in SBL and CHL than in their respective controls and is in accord with the increased DA (Figure 1b) and pH change (Figure 1c).

The mean ratios at maximal counts were <1.5 in SBF for all species and the mixed culture and were similar to one another. Ratios were >1.5 in CHF for *B. bifidum* and *B. infantis* because of higher production of AA by these species. The ratios in CHF for *B. longum* and *B. breve* were <1.5 and were similar to each other, but different ($P < 0.05$) from each other for all four species and for the mixed culture (Table 4).

CONCLUSIONS

Bifidogenic factors, lactulose and FOS, did not influence the growth of bifidobacteria in SB and CH in vitro. Lactulose was stimulatory to acid production and changes in pH to *B. infantis* in both SB and CH. Maximal bacterial counts for the mixed culture were similar to those for *B. breve* in SB and CH and were unaffected by lactulose. For *B. breve*, FOS was inhibited in SB and CH after the end of the log phase, which corresponded to the lower DA and lesser change in pH of the formulas. Maximal counts, acid production, and change in pH and biochemical metabolites at maximal bacterial counts were not influenced by the presence of FOS in the infant formulas.

Reports on the beneficial effects of FOS are based on in vivo studies in the absence of simple sugars. Simple sugars and complex carbohydrates provide a source of energy for maintaining growth and viability of bifidobacteria. In this in vitro study, lactulose and FOS were species specific. These complex carbohydrates were not the preferred source of nutrients for bifidobacteria to enhance growth in vitro in infant formula. It would be of interest to conduct in vivo studies to evaluate the effects of these growth factors on survival of bifidobacteria in infants fed formulas that were not based on milk, such as SB and CH.

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