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Short Fractions of Oligofructose Are Preferentially Metabolized by *Bifidobacterium animalis* DN-173 010

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The growth of *Bifidobacterium animalis* DN-173 010 on different energy sources was studied through small- and large-scale fermentations. Growth on both more common energy sources (glucose, fructose, galactose, lactose, and sucrose) and inulin-type fructans was examined. High-performance liquid chromatography analysis was used to investigate the kinetics. Gas chromatography was used to determine the fructan degradation during the fermentation process. *B. animalis* DN-173 010 was unable to grow on a medium containing glucose as the sole energy source. In general, monosaccharides were poor growth substrates for the *B. animalis* strain. The fermentations with the inulin-type fructans resulted in changes in both growth and metabolite production due to the preferential metabolism of certain fructans, especially the short-chain oligomers. Only after depletion of the shorter chains were the larger fractions also metabolized, although to a lesser extent. Acetic acid was the major metabolite produced during all fermentation experiments. At the beginning of the fermentation, high levels of lactic acid were produced, which were partially replaced by formic acid at later stages. This suggests a shift in sugar metabolism to gain additional ATP that is necessary for growth on oligofructose, which is metabolized more slowly.

The human large intestine is a complex ecosystem in which several hundreds of different bacterial species reside (13). This microflora metabolizes nondigested dietary carbohydrates (resistant starch, nonstarch polysaccharides, and other sugars such as lactose, raffinose, and stachyose) to a variety of products such as short-chain fatty acids (e.g., acetic acid, propionic acid, and butyric acid), other organic acids (e.g., lactic acid, succinic acid, and pyruvic acid), and gases (e.g., H_2, H_2S, CO_2, and CH_4). Bifidobacteria are a predominant group of the colonic microflora that can account for up to 25% of the total number of bacteria present (23). Due to their heterofermentative nature, bifidobacteria can produce lactic acid and ethanol as well as several short-chain fatty acids such as acetic acid and formic acid. Some investigators also mention the production of small amounts of carbon dioxide and succinic acid by *Bifidobacterium* strains (1). The shift in intestinal pH induced by the acidic metabolites during carbohydrate fermentation inhibits the growth of undesirable, potentially pathogenic bacteria (11). Bifidobacteria can also confer several other benefits on their host, such as vitamin production (40), cholesterol-lowering effects (39), immunostimulating effects (8, 46), anticarcinogenic activity (35), a decrease of intestinal transit time (24), and protection against infections (6, 14). This indicates that an increase in the number of bifidobacteria in the human large intestine, due to probiotic, prebiotic, or synbiotic action, will positively affect human health (3).

Probiotics, prebiotics, and synbiotics are considered the main dietary products marketed under the category of functional foods; these are foods with an added health value above their nutritional properties (38). Probiotics can be defined as live microorganisms which on ingestion in certain numbers exert health benefits beyond inherent basic nutrition (15). To overcome difficulties associated with the use of probiotics, such as the necessity for viability and transitory adhesion of the cells to exert a health effect, the use of prebiotics to increase the number of desirable endogenous bacteria has been introduced. A prebiotic can be defined as a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, thus improving host health (12). The most common examples of prebiotics for bifidobacteria are inulin and oligofructose, although several others exist (30). These prebiotics stimulate the specific growth of bifidobacteria, which is the so-called bifidogenic effect (37, 44), but strain and species differences occur in bifidobacterial carbohydrate utilization patterns (4, 16, 22). Finally, a synbiotic is a combination of a probiotic and a prebiotic, in which the prebiotic is used to increase the intestinal survival of the health-promoting bacteria with the ultimate goal of modifying the gut flora and its metabolism (9).

Inulin and oligofructose are two examples of fructans of the inulin type. Inulin-type fructans are linear D-fructose polymers linked by β(2-1)-glycosidic bonds, often with a terminal glucose moiety that is linked by an α(1-2)-glycosidic bond, as in sucrose. The degree of polymerization (DP) of oligofructose varies between 2 and 10, whereas that of inulin can be 60 or even more (36). The β(2-1) linkages of these fructans prevent their digestion in the upper part of the human gastrointestinal tract and are responsible for their reduced caloric value and dietary fiber-like effects (37). Once they arrive in the colon, these fructans are selectively metabolized by bifidobacteria, producing β-fructofuranosidases that hydrolyze these bonds (45). Inulin and oligofructose are industrially produced either

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enzymatically through fructosyltransferase action on sucrose or by extraction from specific plants or plant parts such as chicory (*Cichorium intybus*) roots. Inulin and oligofructose derived from chicory roots contain both fructose chains (F_m) and fructose chains with a terminal glucose unit (GF_n). Synthetic oligofructose contains only fructose chains with glucose end units (5). Data on the kinetics of bifidobacteria grown on inulin-type fructans are scarce (7, 16, 28, 29).

The aim of this study was to examine the kinetics of a dairy probiotic *Bifidobacterium* strain of commercial importance with different carbohydrates, in particular several inulin-type fructans. Furthermore, this study was carried out to obtain quantitative data about the degradation of the inulin-type fructans throughout the fermentation process.

MATERIALS AND METHODS

Microorganism and media. The *Bifidobacterium animalis* strain DN-173 010 used throughout this study was obtained from Danone Vitapole (Palaiseau, France). It was stored at −80°C in modified LAPT (mLAPT) medium (33) with lactose (20 g liter^{-1}), supplemented with 25% (vol/vol) glycerol. mLAPT medium contained bacteriological peptone (Oxoid, Basingstoke, United Kingdom), 15 g liter^{-1}; yeast extract (VWR International, Darmstadt, Germany), 10 g liter^{-1}; tryptone (Oxoid), 10 g liter^{-1}; L-cysteine hydrochloride, 0.5 g liter^{-1}; and Tween 80, 1 ml liter^{-1}. The pH of the medium was adjusted to 6.5 before sterilization (121°C for 20 min). mLAPT medium was also used as the fermentation medium. Lactose, glucose, fructose, galactose, sucrose, or inulin-type fructans (Raftilose P95, Raftiline HP, or Raftilose Synergy1) were used as energy sources (at 20 g liter^{-1} unless stated otherwise). The energy sources, except for the inulin-type fructans, were autoclaved separately and added aseptically to the mLAPT medium. Inulin-type fructans were filter sterilized using VacuCap 60 filters (pore size, 0.20 μm; Pall Gelman Laboratory, Ann Arbor, Mich.).

Lactose, glucose, fructose, galactose, and sucrose were purchased from VWR International, and Raftiline HP, Raftilose P95, and Raftilose Synergy1 were kindly provided by ORAFTI N.V. (Tienen, Belgium). Raftiline HP is a commercial powder containing chicory inulin (>99.5%, wt/wt), and a little glucose, fructose, and sucrose. The average DP of the inulin chains exceeds 23 due to removal of the smaller molecules. Raftilose P95 is a commercial powder produced through enzymatic hydrolysis of chicory inulin. The powder contains oligofructose (>93.2%, wt/wt) with a little glucose, fructose, and sucrose. The DP of the oligofructose chains varies between 2 and 8, with an average of 4. Raftilose Synergy1 is a commercial powder containing oligofructose and inulin (90 to 94%, wt/wt), glucose and fructose (4 to 6%, wt/wt), and sucrose (2 to 4%, wt/wt).

Fermentation experiments. To investigate the influence of different energy sources on the growth of *B. animalis* DN-173 010, small-scale fermentations in glass bottles (100 ml) containing mLAPT medium were carried out at 37°C in duplicate. Incubations took place anaerobically in a Modular Atmosphere Controlled System (MACS; MG Anaerobic Work Station, Don Withley Scientific, Ltd., Shipley, United Kingdom) that was continuously sparged with a mixture of 80% N_2, 10% CO_2, and 10% H_2 (Air Liquide, Paris, France). The inoculum buildup was carried out as follows. First, 10 ml of the mLAPT medium with lactose as the sole energy source was inoculated with a small amount of stock culture (−80°C). After 24 h of incubation, 1 ml was transferred into 10 ml of mLAPT medium supplemented with the energy source to be used afterward. Three such subcultivations were performed after 48, 17, and 12 h of incubation, respectively, to allow the strain to adapt to the energy source chosen. Finally, after another 12 h of incubation, 10 ml was transferred into 100 ml of the final medium. The mLAPT media with Raftiline HP, fructose, or glucose as the sole energy source were inoculated with a preculture grown on lactose, because of the very poor growth of the strain on these three carbohydrates. To build up an inoculum on galactose, only one incubation step of 41 h was performed because of the poor growth of the strain with this energy source. During the fermentations, samples were withdrawn at regular time intervals to measure the optical density (OD).

Kinetic analysis of the growth of *B. animalis* DN-173 010 in mLAPT medium with lactose and inulin-type fructans was carried out on a 5- or 10-liter scale in duplicate. The 10-liter fermentations (2% [wt/vol] lactose, 1 and 2% [wt/vol] Raftilose P95, and 2% [wt/vol] Raftilose Synergy1) were carried out in a Biostat C fermentor (B. Braun Biotech International GmbH, Melsungen, Germany). The 5-liter fermentation (1% [wt/vol] Raftilose Synergy1) was carried out in a Biostat CT fermentor (B. Braun Biotech International GmbH). The inoculum buildup was carried out as follows. A 10-ml volume of mLAPT medium with the final energy source was inoculated with a small amount of stock culture (−80°C). After 16 h of anaerobic incubation at 37°C, 2 ml was transferred into 20 ml of mLAPT medium containing the final energy source. After 12 h of incubation, this 20 ml was used to inoculate 200 ml of the final mLAPT medium. After another 12 h of incubation, the latter culture was used to inoculate the fermentor. For the 5-liter fermentation with 1% (wt/vol) Raftilose Synergy1, an inoculum of 100 ml was used. The fermentations with Raftiline HP and fructose were inoculated with a lactose-grown preculture due to the poor growth on these carbohydrates. All fermentations were carried out anaerobically by sparging the medium with a mixture of 90% N_2 and 10% CO_2 (Air Liquide) at a constant pH of 6.5 ± 0.05 that was controlled through automatic addition of 10 N NaOH and at a temperature of 37 ± 0.1°C. Samples were withdrawn at regular time intervals for further analysis.

Analysis of microbial growth. To determine cell growth, the OD_{600} of the samples, appropriately diluted if necessary, was measured twice. Cell numbers (CFU milliliter^{-1}) were obtained through plating on mLAPT medium with lactose, supplemented with 1.5% (wt/vol) agar (Oxoid). The incubation was carried out anaerobically (MACS) at 37°C for 48 h. The cell dry mass was obtained through filtration of a fixed volume of sample. The filters (HAWP; pore size, 0.45 μm; Millipore, Bedford, Mass.) were dried at 105°C for 24 h and weighed.

Analysis of metabolites. The amounts of sugars, lactic acid, acetic acid, formic acid, and ethanol were determined by high-performance liquid chromatography (HPLC) analysis with a Waters chromatograph (Waters Corp., Milford, Mass.), equipped with a differential refractometer, a controller, a column oven, and an autosampler. A Polyspher OAKC column (VWR International) was used with 5 mN H_2SO_4 as the mobile phase at a flow rate of 0.4 ml min^{-1}. The column temperature was kept at 35°C. Samples were centrifuged (16,060 × *g* for 15 min), and an equal volume of 20% (vol/vol) trichloroacetic acid was added. After centrifugation (16,060 × *g* for 15 min), the supernatant was filtered (nylon syringe filters; pore size, 0.45 μm; Euroscientific, Lint, Belgium) before injection. Due to acid hydrolysis caused by trichloroacetic acid, the total fructan concentration was measured as the amount of fructose. However, this acid hydrolysis was incomplete. Therefore, errors of up to 10% for the total fructan concentration measured by HPLC occurred. The errors in the measurements of glucose, galactose, fructose, lactose, acetic acid, lactic acid, formic acid, and ethanol were 0.40, 0.88, 0.59, 1.96, 2.29, 0.44, 1.40, and 1.01%, respectively.

Quantitative analysis of fructans was carried out by gas chromatography as described previously (19).

The maximal specific growth rate, μ_{max}, the cell yield coefficient, $Y_{X/S}$, and the product yield coefficient, $Y_{P/S}$, were determined by plotting ln (X/X_0) as a function of time, $(X - X_0)$ as a function of $(S_0 - S)$, and $(P - P_0)$ as a function of $(S_0 - S)$, respectively, where X_0, S_0, and P_0 refer to the biomass concentration (grams of cell dry mass liter^{-1}), the substrate concentration (grams liter^{-1}), and the total metabolite concentration (moles liter^{-1}) at the beginning of the fermentation, respectively.

RESULTS

Growth in mLAPT medium. All experiments were carried out with mLAPT medium containing an appropriate energy source, because growth of the strain in mLAPT medium without an added energy source was not possible. In addition, all large-scale fermentations were carried out at constant pH to avoid growth inhibition of the *Bifidobacterium* strain due to the pH decrease generated during fermentation. A constant pH of 6.5 was chosen because this is the average pH of the colon.

Influence of different energy sources on the growth of *B. animalis* DN-173 010. The monosaccharides glucose, fructose, and galactose were not fermented, although at the end of the fermentation with galactose the OD_{600} increased slightly (Fig. 1). To support this observation, several media were tested, but it was found that the strain could not grow until the monosaccharides were replaced by lactose (results not shown). The disaccharides lactose and sucrose were fermented within a reasonable time (<24 h), although the fermentation with su-

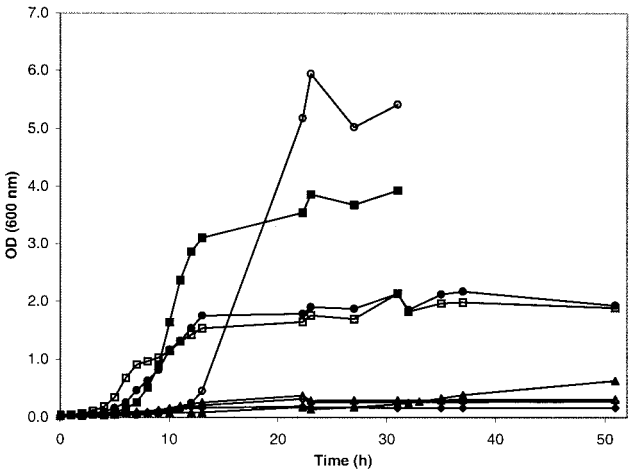

FIG. 1. Small-scale fermentations of *B. animalis* DN-173 010 on different carbohydrates (2% [wt/vol]). ●, Raftilose P95; ○, sucrose; ■, lactose; □, Raftilose Synergyl; ▲, galactose; △, glucose; ◆, Raftiline HP; ◇, fructose. The graph is representative of the results of two experiments.

crose showed a lag phase of about 8 h. The inulin-type fructans Raftilose P95 and Raftilose Synergy1 were fermented from the start of the fermentation. The fermentation pattern of Raftilose Synergy1 showed several changes in growth behavior. Raftiline HP was not fermented. With respect to final OD_{600}, lactose and sucrose were good energy sources whereas Raftilose P95 and Raftilose Synergy1 supported less growth.

Fermentation course of *B. animalis* DN-173 010 on lactose and inulin-type fructans. The fermentation with 2% (wt/vol) lactose showed the best growth of *B. animalis* DN-173 010 (Fig. 2). During this fermentation, galactose and glucose accumulated in the medium, possibly due to extracellular hydrolysis. After depletion of lactose, extracellular galactose was used as an energy source. Finally, after galactose depletion, extracellular glucose was used but to a lesser extent. However, galactose and glucose were used mainly for maintenance because of their consumption, despite a decrease of OD_{600}. During the consumption of galactose and glucose, an increase in formic acid production was observed while the production of lactic acid stopped. Formic acid production was low during growth on lactose. The production of acetic acid was always high, and its concentration was higher than that of lactic acid or formic acid.

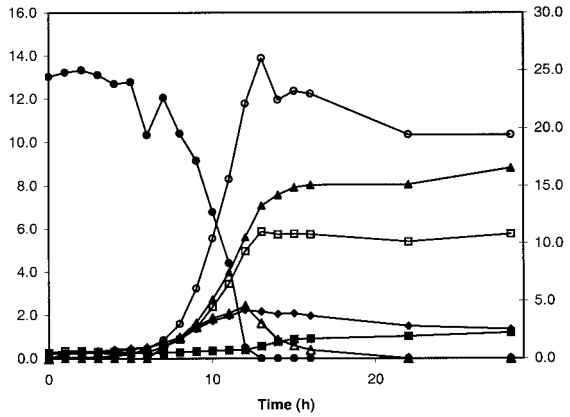

FIG. 2. Fermentation of *B. animalis* DN-173 010 with 2% (wt/vol) lactose. Right axis: ●, lactose concentration (g liter^{-1}). Left axis: ○, OD_{600}; ■, formic acid concentration (g liter^{-1}); □, lactic acid concentration (g liter^{-1}); ▲, acetic acid concentration (g liter^{-1}); △, galactose concentration (g liter^{-1}); ◆, glucose concentration (g liter^{-1}). The graph is representative of the results of two experiments.

The fermentations with fructose and Raftiline HP showed little growth at the early stage of the fermentation (0 to 5 h), but later in fermentation no growth could be detected (results not shown). The minor growth was probably due to the presence of residual lactose carried over by the inoculum.

The fermentations with 1% (wt/vol) (Fig. 3a) and 2% (wt/vol) (Fig. 3b) Raftilose P95 showed a similar growth pattern. Both fermentations displayed a switch in growth behavior, resulting in exponential growth followed by linear growth. For the fermentation with 1% Raftilose P95, linear growth started later. During exponential growth, lactic acid and acetic acid were the main metabolites produced. The linear growth resulted in an increase in formic acid production and a retardation of lactic acid production; the acetic acid production remained high. The fermentations with Raftilose P95 were the only ones where ethanol production could be detected at the end of the fermentation.

The fermentations with 1% (wt/vol) (Fig. 4a) and 2% (wt/vol) (Fig. 4b) Raftilose Synergy1 were also very similar. Both fermentations displayed two changes in growth behavior, resulting in both exponential growth (twice) and linear growth. The linear growth started later for the 1% fermentation. During the first exponential growth stage, only lactic acid production was observed. At the end of the second exponential growth stage, formic acid production started. Acetic acid was the main metabolite produced throughout.

Kinetic analysis of the growth of *B. animalis* DN-173 010 on lactose and inulin-type fructans. The kinetic parameters of the fermentations are summarized in Table 1. The maximum specific growth rate (μ_{max}) and the cell yield coefficient ($Y_{X/S}$) during the first exponential growth stage were comparable for all fermentations: μ_{max} varied between 0.53 and 0.69 h^{-1}, and $Y_{X/S}$ varied between 0.17 and 0.31 g of CDM g of substrate^{-1}. The production of metabolites differed for the exponential and linear growth. Acetic acid production was almost always high. Lactic acid production was always high in the early stage of fermentation and decreased later in most cases. In contrast, formic acid production was low during the first few hours of fermentation but increased later. The maximum concentration of formic acid varied from 0.96 ± 0.01 to 2.46 ± 0.03 g liter^{-1}. During linear growth during the fermentations with Raftilose Synergy1, lactic acid, acetic acid, and formic acid were produced. The total product yield coefficient ($Y_{P/S}$) was always lower for the first exponential growth stage than for successive growth. The molar ratio of acetic acid to lactic acid at the end of the fermentation process varied between 2.4 (fermentation with 2% [wt/vol] lactose) and 11.6 (fermentation with 1% [wt/vol] Raftilose P95), which differs considerably from the theoretical value of 1.5. A very low production of lactic acid was observed for the fermentation with 1% (wt/vol) Raftilose P95 and with 1% (wt/vol) Raftilose Synergy1. For these fermentations, formic acid production largely replaced lactic acid pro-

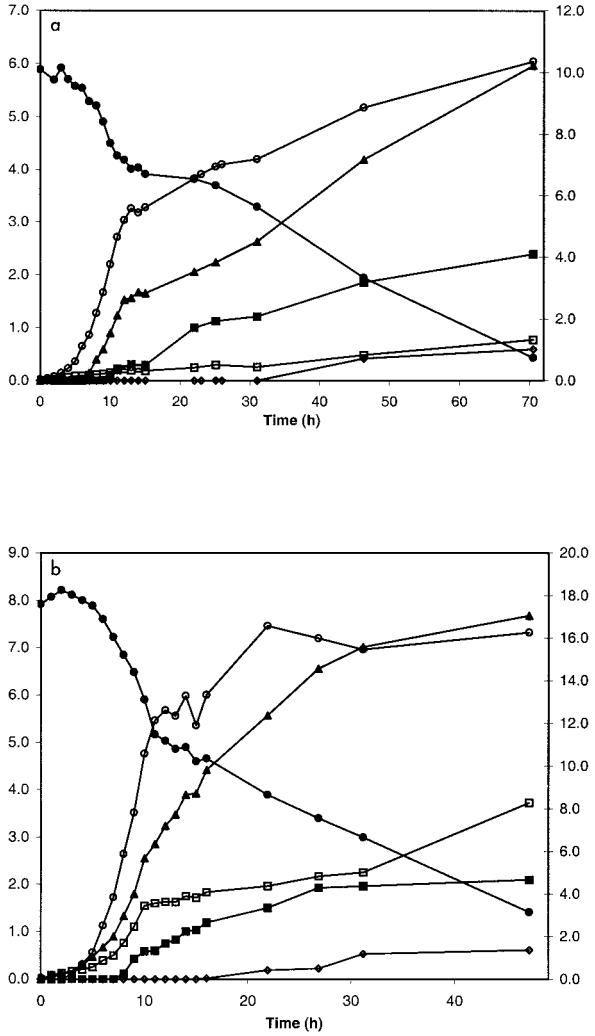

FIG. 3. (a) Fermentation of *B. animalis* DN-173 010 with 1% (wt/vol) Raftilose P95. Right axis: ●, fructose concentration (g liter^{-1}). Left axis: ○, OD_{600}; ■, formic acid concentration (g liter^{-1}); □, lactic acid concentration (g liter^{-1}); ▲, acetic acid concentration (g liter^{-1}); ◇, ethanol concentration (g liter^{-1}). The graph is representative of the results of two experiments. (b) Fermentation of *B. animalis* DN-173 010 with 2% (wt/vol) Raftilose P95. Right axis: ●, fructose concentration (g liter^{-1}). Left axis: ○, OD_{600}; ■, formic acid concentration (g liter^{-1}); □, lactic acid concentration (g liter^{-1}); ▲, acetic acid concentration (g liter^{-1}); ◇, ethanol concentration (g liter^{-1}). The graph is representative of the results of two experiments.

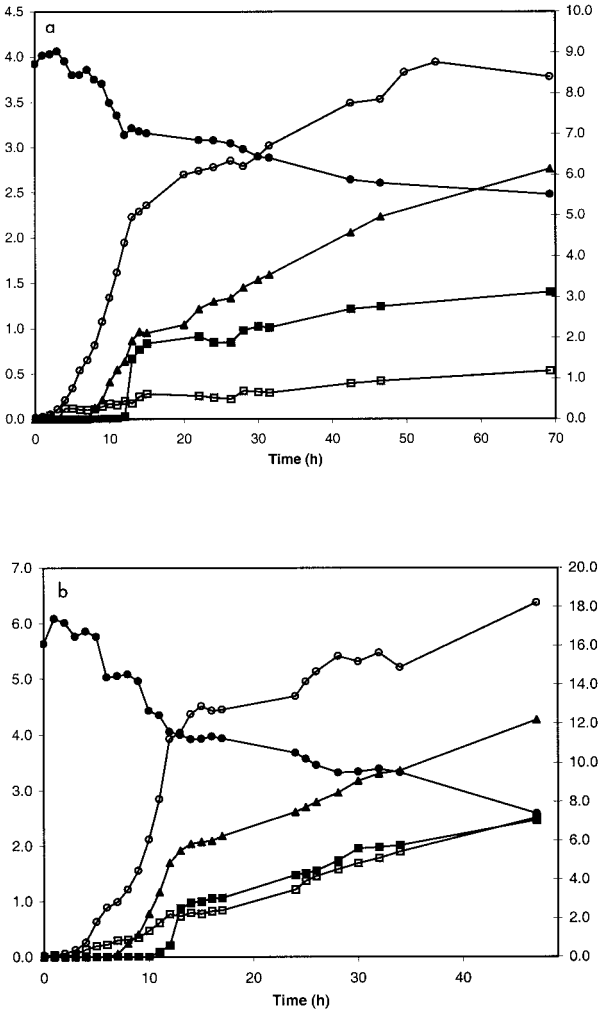

FIG. 4. (a) Fermentation of *B. animalis* DN-173 010 with 1% (wt/vol) Raftilose Synergyl. Right axis: ●, fructose concentration (g liter^{-1}). Left axis: ○, OD_{600}; ■, formic acid concentration (g liter^{-1}); □, lactic acid concentration (g liter^{-1}); ▲, acetic acid concentration (g liter^{-1}). The graph is representative of the results of two experiments. (b) Fermentation of *B. animalis* DN-173 010 with 2% (wt/vol) Raftilose Synergyl. Right axis: ●, fructose concentration (grams liter^{-1}). Left axis: ○, OD_{600}; ■, formic acid concentration (g liter^{-1}); □, lactic acid concentration (g liter^{-1}); ▲, acetic acid concentration (g liter^{-1}). The graph is representative of the results of two experiments.

duction, resulting in a very low molar ratio of lactic acid to formic acid, of 0.17 and 0.14 mol of lactic acid mol of formic acid^{-1}, respectively. The molar ratio of acetic acid to formic acid was higher for the fermentation with lactose than for the fermentations with inulin-type fructans. When ethanol was produced, the production of formic acid was lowered.

Fructan analysis. Gas chromatography analysis of the different fructan chains was performed in detail for the fermentation with 2% (wt/vol) Raftilose P95 (Table 2) and 2% (wt/vol) Raftilose Synergy1 (Table 3).

Analysis of the Raftilose P95 samples showed the degradation of the F_3 fraction in the early stage of the fermentation (0 to 9 h). Afterward, all the other fractions (especially F_4, GF_4, F_5, GF_5, and F_6) were also metabolized. At the end of the fermentation (47 h), almost all the oligofructose was metabolized (± 1 g of residual oligofructose liter^{-1} [Table 2]). During the fermentation, fructose and glucose accumulated in the medium due to the breakdown of the oligofructose chains. The amounts of sucrose were very small, and consumption of sucrose was hardly observed. The same trend was observed for two strains of *Bifidobacterium longum* (Bb 536 and LMG 10497) when grown in a medium containing oligofructose as the sole energy source (unpublished results). Compared to the *B. animalis* strain DN-173 010, the only difference observed was the rapid consumption of all glucose and fructose that was released into the medium due to breakdown of the fructans.

TABLE 1. Summary of the fermentation parameters for the growth of *B. animalis* DN-173 010 on different carbohydrates[a]

Parameter[b]	Lactose	1% Raftilose	2% Raftilose	1% Synergyl	2% Synergyl
OD_{max}	12.4	6.0	7.5	3.9	6.4
CDM_{max}	5.4	1.8	2.2	1.2	2.1
CFU_{max}	2.9×10^9	2.1×10^9	6.6×10^8	1.8×10^9	2.8×10^9
First exponential growth					
μ_{max}	0.63 (0.99)	0.53 (0.99)	0.64 (0.99)	0.68 (0.99)	0.69 (0.99)
$Y_{X/S}$	0.20 (0.96)	0.30 (0.99)	0.22 (0.98)	0.22 (0.92)	0.31 (0.99)
$Y_{P/S}$	0.0063 (0.96)	0.0089 (0.87)	0.012 (0.99)	0.0029 (0.68)	0.0010 (0.78)
Linear growth (Raftilose) or second exponential growth (Synergyl)					
μ_{max}				0.23 (0.99)	0.28 (0.99)
$Y_{X/S}$		0.25 (0.98)	0.17 (0.98)	0.30 (0.80)	0.32 (0.94)
$Y_{P/S}$		0.022 (0.99)	0.017 (0.93)	0.047 (0.81)	0.022 (0.93)
Linear growth					
$Y_{X/S}$				0.23 (0.63)	0.27 (0.73)
$Y_{P/S}$				0.031 (0.97)	0.021 (0.89)
End of the fermentation					
A/L	2.4	11.6	3.1	7.8	2.6
A/F	7.1	1.9	2.8	1.1	1.1
L/F	2.9	0.17	0.91	0.14	0.43

[a] Numbers in parentheses are correlation coefficients.

[b] OD_{max}, maximal observed OD_{600}; CDM_{max}, maximal observed CDM (in grams of CDM liter^{-1}; standard deviation was 0.03 g of CDM liter^{-1}); CFU_{max}, maximal observed number of CFU milliliter^{-1}; μ_{max}, maximal specific growth rate (hours^{-1}); $Y_{X/S}$, cell yield coefficient (grams of CDM gram of substrate^{-1}); $Y_{P/S}$, product yield coefficient (moles of metabolites gram of substrate^{-1}); A/L, moles of acetic acid mole of lactic acid^{-1}; A/F, moles of acetic acid mole of formic acid^{-1}; L/F, moles of lactic acid mole of formic acid^{-1}.

Analysis of the Raftilose Synergy1 samples (Table 3) revealed the presence of larger amounts of sucrose than those found with the Raftilose P95 samples. This sucrose was metabolized during the first hours of fermentation (0 to 6 h). To support these results, a preculture grown on Raftilose Synergy1 was used to inoculate mLAPT medium with sucrose as the sole energy source. No lag phase was observed (results not shown), whereas a preculture grown on sucrose showed a lag phase of 8 h (Fig. 1). No data about the different inulin fractions were obtained. However, the average DP increased on further fermentation. Glucose and fructose were not metabolized during the first 16 h. After 30 h of fermentation, the small

TABLE 2. Fructan degradation during the growth of *B. animalis* DN-173 010 on oligofructose as 2% (wt/vol) Raftilose P95

Component[a]	Concn (g 100 ml^{-1}) at time (h):							
	0	3	6	9	11	14	22	47
F	0.07	0.07	0.08	0.16	0.20	0.25	0.37	0.31
G	0.02	0.02	0.02	0.03	0.03	0.04	0.07	0.08
GF	0.006	0.008	0.001	0.003	0.004	0.002	0.004	0.003
F_2	0.06	0.06	0.05	0.05	0.02	0.01	0.01	0.007
GF_2	0.006	0.008	0.006	0.01	0.02	0.01	0.02	0.004
F_3	0.53	0.56	0.45	0.25	0.09	0.07	0.04	0.003
GF_3	0.06	0.06	0.06	0.07	0.08	0.08	0.07	0.006
F_4	0.56	0.56	0.54	0.54	0.49	0.37	0.17	0.008
GF_4	0.14	0.14	0.14	0.15	0.15	0.13	0.10	0.01
F_5	0.23	0.24	0.23	0.25	0.26	0.23	0.18	0.02
GF_5	0.12	0.12	0.11	0.13	0.14	0.13	0.11	0.01
F_6	0.16	0.16	0.15	0.17	0.19	0.16	0.13	0.01
GF_6	0.06	0.06	0.06	0.07	0.07	0.06	0.05	0.006
F_7	0.04	0.05	0.04	0.05	0.05	0.05	0.04	0.005
$GF_7 + F_8$	0.006	0.007	0.006	0.006	0.007	0.007	0.01	0.007
F_8	0.006	0.007	0.006	0.006	0.009	0.007		
$GF_8 + F_9$	0.003	0.004	0.005	0.007	0.01	0.007	0.006	0.005
F_9	0.003	0.004						
DP_{10}	0.001	0.003	0.002	<0.01	0.002	0.002	0.002	0.001
Oligofructose	2.0	2.0	1.9	1.8	1.6	1.3	0.9	0.1

[a] F, fructose; G, glucose; DP_{10}, degree of polymerization of 10.

TABLE 3. Fructan degradation during the growth of *B. animalis* DN-173 010 on a mixture of inulin and oligofructose as 2% (wt/vol) Raftilose Synergyl

Component[a]	Concn (g 100 ml^{-1}) at time (h):							
	0	3	6	9	12	16	30	47
Fructose	0.08	0.08	0.09	0.09	0.09	0.10	0.006	0.009
Glucose	0.03	0.03	0.03	0.03	0.03	0.03	0.002	0.001
Sucrose	0.07	0.06	0.002	0.001	0.001	0.001	0.0007	0.001
Fructan	2.1	2.2	2.2	2.1	2.0	1.9	1.9	1.6
DP_{av}	16.6	17.3	17.2	17.8	19.2	19.5	20.2	21.6

[a] DP_{av}, average degree of polymerization of the remaining fructans.

amounts of glucose and fructose that were present in the medium were also metabolized.

DISCUSSION

It is well known that sugar metabolism by lactic acid bacteria is species and even strain dependent (31). Many differences in prebiotic fermentation patterns are described for lactic acid bacterial strains, in particular for probiotic *Bifidobacterium* strains (2, 4, 16, 22, 36). Therefore, when a certain probiotic strain is combined with a prebiotic into a synbiotic, a detailed study of their interaction is required. However, data on the kinetics of bifidobacteria grown on inulin-type fructans are scarce (7, 16, 28, 29).

The inability of *B. animalis* DN-173 010 to metabolize glucose is most remarkable. The small-scale fermentations showed that not only glucose but also fructose and galactose were very poor substrates for this strain. The fact that the di- and oligosaccharides (lactose, sucrose, and oligofructose) were metabolized faster than their constituting moieties (glucose, fructose, and galactose) suggests that this strain lacks the necessary uptake systems for these monosaccharides. Furthermore, it is possible that the enzymes necessary for the breakdown of di- and oligosaccharides can be induced, because changes in both growth and metabolite production occur during fermentation with this strain. Such induction of β-fructofuranosidase has been described for *B. infantis* (29). Moreover, β-fructofuranosidase is capable of hydrolyzing the β(2-1) and α(1-2) linkages of inulin and sucrose, respectively (18). The easy fermentation of lactose indicates the adaptation of this industrial strain to a milk medium. Most probably, β-galactosidase is produced constitutively. To our knowledge, this is the first report of a strain of *B. animalis* unable to grow on a medium with glucose as the sole energy source, although other glucose-nonfermenting *Bifidobacterium* strains have been reported previously (32). The adaptation of the strain to metabolize di- and oligosaccharides can be an advantage for the strain in its competition with other microorganisms in the human gut, where oligo- and polysaccharides are the main sugars.

Fermentation with lactose showed the best growth compared with fermentations with Raftilose P95 or Raftilose Synergy1 (poor growth) and fermentations with fructose or Raftiline HP (no growth). Fructose or large fructose polymers were not metabolized at all, for which missing uptake systems or the absence of the appropriate enzymes for their breakdown may be responsible. Oligofructose was metabolized preferentially, indicating that uptake most probably takes place first and that the uptake of short-chain fructans induces the enzymes necessary for β(2-1) hydrolysis and subsequent metabolism of fructose monomers (21). Similar results were obtained for the growth of a *B. adolescentis* strain on galactooligosaccharides (43) and for the growth of a *B. infantis* strain on fructooligosaccharides (29). Other studies also mentioned the inability of some strains of *Bifidobacterium* to metabolize the larger fructan polymers (2, 4, 16, 22). The inability of the *B. animalis* DN-173 010 strain to metabolize the larger fructan chains is interesting from a commercial point of view in creating a product, containing both a probiotic and a prebiotic, in which the probiotic strain does not affect the prebiotic during storage and transport. Once consumed, both the probiotic and the prebiotic may exert their beneficial health effects in the human colon.

The fermentations with Raftilose P95 showed changes in both growth and metabolite production, thereby indicating that some oligofructose fractions were preferentially metabolized. Quantitative analysis demonstrated the consumption of the F_3 fraction in the early stage of fermentation, resulting in exponential growth, while other fractions were metabolized later, resulting in linear growth. The latter was also observed for the growth of two strains of *B. longum* in media containing oligofructose as the sole energy source (unpublished results). Comparable results have been reported previously (20, 29, 43). In general, utilization of fructooligosaccharides with a low DP is enhanced in bifidobacteria (22, 34, 36). The primary exponential growth was probably necessary to obtain enough biomass to produce sufficient amounts of enzymes, such as invertase or β-fructofuranosidase (26, 29), which are indispensable for the rapid breakdown of the other fractions. The delay of the linear growth with the fermentation of 1% (wt/vol) Raftilose P95 compared with the 2% (wt/vol) Raftilose P95 fermentation could be explained by the higher sugar concentration in the latter, since this allows a larger amount of biomass and hence of necessary enzymes to be produced during exponential growth. This resulted in a faster breakdown of the longer chains and an earlier start of the linear growth. The same was observed with the Raftilose Synergy1 fermentations. Here, sucrose that is present as a contaminant was metabolized from the start, resulting in early exponential growth. This is in contrast with the small-scale fermentations, during which sucrose was metabolized only after a lag phase of approximately 8 h. The rapid consumption of sucrose with the Raftilose Synergy1 fermentations can probably be explained by the presence or the de novo synthesis of invertase or β-fructofurano-

sidase, whose production was stimulated through the inoculum buildup on Raftilose Synergy1. The induction of sucrose utilization genes by oligofructose was recently reported (42). The consecutive consumption of different chain fractions of the fructans by bifidobacteria is interesting for the development of prebiotics that may act in different parts of the human colon, hence influencing the carbohydrate and protein fermentation patterns throughout the colon.

The amounts and ratios of metabolites formed differed for each fermentation and for each growth pattern within a fermentation. Although the total product yield coefficient may display a certain deviation due to the errors of the fructan determination through HPLC, it is obvious that slower growth resulted in the production of larger amounts of metabolites ($Y_{P/S}$ was always lower during the first exponential growth stage compared with successive growth). Acetic acid was the major metabolite produced in all cases. It is indeed well known that acetic acid is the predominant metabolite produced by bifidobacteria, resulting in a theoretical acetic acid-to-lactic acid molar ratio of 1.5 when grown on glucose (1). The production of lactic acid was always high in the early stage of fermentation. Later, this lactic acid production decreased in most cases. However, formic acid production was low at the beginning and high at the end of the fermentation processes. These results indicate a change in the metabolism of the *Bifidobacterium* strain during fermentation of inulin-type fructans. The same trend was observed for *B. longum* Bb 536 when grown in media containing inulin-type fructans as the sole energy source (unpublished results). It may be assumed that well fermented sugars and concomitantly a high intracellular sugar concentration stimulate lactic acid production. On the other hand, a less fermentable sugar and a low intracellular sugar concentration could stimulate formic acid production to produce extra ATP, which is necessary for growth on sugars that are metabolized slowly. Since more reducing power is needed at high intracellular sugar concentrations, pyruvate is converted to lactic acid through the conventional catabolic route to equilibrate the redox balance (1, 25). In *Lactococcus lactis* for instance, formic acid production is observed for growth on more slowly fermentable sugars, such as galactose, and during cultivation at low growth rates in glucose-limited chemostat cultures (41). The regulation of the shift from homolactic formation (i.e., lactic acid is the predominant end product) to mixed-acid product formation (i.e., formic acid, acetic acid, and ethanol production) has been explained by the allosteric modulation of enzymes competing for pyruvate under anaerobic conditions and their rate of transcription (27).

The production of formic acid, and in some cases ethanol, influenced the theoretical molar ratio of acetic acid to lactic acid. The increased production of acetic acid and formic acid may be of interest for the inhibition of intestinal pathogens such as *Escherichia coli* and *Salmonella*. It is indeed well known that the inhibitory potential of bifidobacteria depends on their production of organic acids (10, 17).

To conclude, it has been shown that *B. animalis* DN-173 010 was unable to grow on a medium with glucose or large fructan polymers (DP $>$ 20) as the sole energy sources. The fermentations with oligofructose (Raftilose P95) and a mixture of inulin and oligofructose (Raftilose Synergy1) showed changes in both growth and metabolite production due to the preferential metabolism of shorter fructan fractions over the longer chains. Acetic acid was the main metabolite produced for all energy sources tested. Lactic acid production was high at the beginning of the fermentations. Later, formic acid production occurred at the expense of lactic acid production. This kinetic study will support biochemical and molecular analyses and will help us to understand the interactions between this probiotic *Bifidobacterium* strain and different commercial products containing inulin-type fructans. The understanding of the interactions between probiotics and prebiotics will facilitate the rational development of synbiotics.

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