Exopolysaccharide and Kestose Production by *Lactobacillus sanfranciscensis* LTH2590

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Received 16 August 2002/Accepted 19 January 2003

The effect was investigated of sucrose concentration on sucrose metabolism and on the formation of exopolysaccharide (EPS) by *Lactobacillus sanfranciscensis* LTH2590 in pH-controlled fermentations with sucrose concentrations ranging from 20 to 160 g liter⁻¹. The EPS production increased and the relative sucrose hydrolysis activity decreased by increasing the sucrose concentration in the medium. The carbon recovery decreased from 95% at a sucrose concentration of 30 g liter⁻¹ to 58% at a sucrose concentration of 160 g liter⁻¹ because of the production of an unknown metabolite by *L. sanfranciscensis*. This metabolite was characterized as a fructo-oligosaccharide. The oligosaccharide produced by *L. sanfranciscensis* was purified and characterized as a trisaccharide with a glucose/fructose ratio of 1:2. The comparison of the retention time of this oligosaccharide and that of pure oligosaccharide standards using two different chromatography methods revealed that the oligosaccharide produced by *L. sanfranciscensis* LTH2590 is 1-kestose. Kestose production increased concomitantly with the initial sucrose concentration in the medium.

Polysaccharides of plant origin have been used for a long time in the food industry as biothickeners, texture stabilizers, or gelling agents. In the last decades several microbial exopolysaccharides (EPS) have been described as alternatives for plant polysaccharides. Microbial polysaccharides have rheological properties that match the industrial demands and can be produced in large amounts and at high purities. The interest of the food industry in developing multifunctional additives that not only provide the desired improvement of the texture but also have additional nutritional properties led to extensive search for polysaccharides with prebiotic attributes. Prebiotics are nondigestible food ingredients that affect the host beneficially by selectively stimulating the growth and/or activity of specific bacteria in the colon and thus improve host health (12). Fructo-oligosaccharides (FOS), xylo-oligosaccharide, and inulin are some of the prebiotics available for human consumption (5, 24). FOS with prebiotic properties (e.g., kestose and nystose) are polymers of D-fructose joined by $\beta(2\rightarrow1)$ linkages and terminated with a D-glucose molecule linked to fructose by an $\alpha(1\rightarrow2)$ bond as in sucrose (22). The degree of polymerization can vary from 2 to 35. FOS with a degree of polymerization of 3 to 5 are called neosugars (30) and can be enzymatically synthesized from sucrose by using fructosyltransferase from *Aspergillus niger* (16, 17). McKellar and Modler (23) showed that the maximum activity of β -fructosidase, responsible for the hydrolysis of inulin-type polysaccharides by bifidobacteria, was observed with neosugars.

Lactic acid bacteria (LAB) are widely used for the production of numerous fermented foods and are generally recognized as safe. Several EPS-producing LAB such as *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactococcus lactis*, and *Streptococcus thermophilus* have been isolated from fermented

dairy products, in particular, from yogurt (2, 8). The majority of EPS synthesized by LAB are heteropolysaccharides composed of repeating units consisting of two or more monosaccharides, mainly galactose, glucose, rhamnose, and fructose. Heteropolysaccharides are typically produced in small amounts up to 2 g liter⁻¹ (7). Furthermore, the ability of LAB to produce homopolysaccharides has been described. *Lactobacillus reuteri* contains strains known to produce homopolysaccharides of the fructan type (31, 32). Korakli et al. (19) reported the production of EPS of the fructan type by *Lactobacillus sanfranciscensis*, and Tieking et al. (29) have recently described the ability of several lactobacilli of sourdough and intestinal origin to produce EPS of the fructan and glucan types.

L. sanfranciscensis belongs to the microbial flora of traditionally prepared wheat and rye sourdoughs (13). The strain *L. sanfranciscensis* LTH2590 was shown to produce EPS from sucrose (19). This EPS is a high-molecular-mass fructan homopolysaccharide of the levan type (6, 19, 26) and is synthesized by a levansucrase homologous to known bacterial levansucrase enzymes (29). In situ production of EPS by *L. sanfranciscensis* LTH2590 during wheat and rye sourdough fermentation (20) and a bifidogenic activity of this EPS were demonstrated (6, 21). The high potential of sourdough lactobacilli to produce EPS during sourdough fermentation may allow the replacement of plant polysaccharides which are currently used in bread production because they improve the textural properties, keepability, and shelf life of bread (1).

Information about biosynthesis, kinetics of EPS formation, and physiological regulation of homopolysaccharide production by lactobacilli is rather scarce. Hestrin et al. (14) named the enzyme responsible for dextran production by *Leuconostoc mesenteroides* dextranase and Van Hijum et al. (33) purified a levansucrase-synthesizing EPS of the levan type from *L. reuteri*. It was the aim of this work to study the metabolism of sucrose as well as the effect of substrate concentration on sucrose metabolism, EPS production, and the biochemical na-

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ture of the respective end products formed by *L. sanfranciscensis*. The strain *L. sanfranciscensis* LTH2590 was chosen because it produces EPS and cannot use fructose as an energy source but reduces it to mannitol, therewith regenerating NADH to NAD⁺ (19).

MATERIALS AND METHODS

Organism and culture conditions. The EPS-producing strain *L. sanfranciscensis* LTH2590 was cultured at 30°C in sucrose-MRS (Su-MRS) containing the following components per liter: peptone from casein, 10 g; yeast extract, 5 g; meat extract, 5 g; K₂HPO₄ · 3H₂O, 2.6 g; KH₂PO₄, 4 g; cysteine-HCl, 0.5 g; NH₄Cl, 3 g; Tween 80, 1 ml. Stock solutions containing MgSO₄ · 3H₂O (100 g liter⁻¹) and MnSO₄ · 4H₂O (50 g liter⁻¹) were autoclaved separately, and a vitamin mix containing folic acid, pyridoxal phosphate, thiamine, riboflavin, cobalamin, and pantothenic acid (0.2 g liter⁻¹ each) was sterilized by filtration. One milliliter of each stock solution liter⁻¹ was added to the sterilized medium. Sucrose and fructose were sterilized by filtration; 20 g of sucrose liter⁻¹ and 15 g of fructose liter⁻¹ were used unless otherwise indicated.

Fermentations at controlled pH were carried out in a 350-ml jar fermentor (Braun Biotech, Inc., Melsungen, Germany) at 30°C and 80 rpm without a gas inlet. The pH was monitored with an online pH sensor and maintained at 5.6 with 4 M NaOH. Medium components were dissolved in 60% of the final volume and autoclaved in the fermentor (20 min at 121°C). Sucrose and fructose were dissolved in the residual volume (40%), sterilized by filtration, and added to the autoclaved medium.

Determination of the maximum growth rate. The effect of sucrose and fructose concentration on the maximum growth rate was determined in multiple-well plates (Sarstedt, Nümbrecht, Germany). Su-MRS with sucrose and fructose concentrations ranging from 0 to 133 g liter⁻¹ and 0 to 53 g liter⁻¹, respectively, was used. The kinetics of growth and maximum growth rates were determined at 590 nm with a spectrophotometer (SpectraFluor; Tecan, Grödig, Austria). The modeling of the maximum growth rate as a function of sucrose and fructose was performed as described previously (11). Results are representative for two independent experiments with consistent results.

Determination of CFU. Cell counts were determined on mMRS4 (27). The appropriate dilution was plated by using a spiral plater (IUL, Königswinter, Germany), and plates were incubated at 30°C for 48 h under a controlled atmosphere (76% N₂, 20% CO₂, 4% O₂).

Determination of metabolites. During pH-controlled fermentations, samples were taken for determination of viable cell counts, optical density, and high-performance liquid chromatography (HPLC) analysis. Cells were removed by centrifugation (14,000 × g for 5 min.). The concentrations of lactic acid, acetic acid, and ethanol in the supernatant were determined with a polyspher OA KC column (Merck, Darmstadt, Germany). The flow rate was 0.4 ml min⁻¹, the mobile phase was 5 mmol of H₂SO₄ liter⁻¹, and the temperature of the column was 70°C. Sucrose, glucose, fructose, kestose, and mannitol concentrations were determined with a polyspher CH PB column (Merck). The flow rate was 0.4 ml min⁻¹, the mobile phase was deionized H₂O, and the temperature of the column was 80°C. A refractive index detector (Gynkotek, Germering, Germany) was used for the detection of organic acids and carbohydrates. Six of nine fermentations were performed in duplicate, and the coefficient of variation of optical density data as well as the concentrations of educts and products of metabolism between two independent fermentations under identical conditions was generally less than 12%.

The retention time of the oligosaccharide produced by *L. sanfranciscensis* LTH2590 was compared with that of kestose by using anion-exchange chromatography coupled to an integrated and pulsed amperometric detection equipped with a gold electrode cell (Dionex, Sunnyvale, Calif.). The waveform was set as described by Jandik et al. (18). The column used was an AminoPac PA10 (Dionex) consisting of a guard column and an analytical column (2 by 250 mm). Three different eluents were used: deionized water (eluent A), 250 mM NaOH (eluent B), and 1 M sodium acetate (eluent C). The water used for the preparation of the eluents had a conductivity of 0.054 μS cm⁻¹. The flow rate was 0.25 ml min⁻¹, and the temperature of the column was 30°C. The following solvent gradient was used to separate sugars and oligosaccharides: 0 min, 80% eluent A, 0% eluent B, and 20% eluent C; 16 min, 80% eluent A, 0% eluent B, and 20% eluent C; 32 min, 0% eluent A, 80% eluent B, and 20% eluent C. Helium was used for degassing the eluents and to prevent the formation of carbonates.

EPS isolation, purification, and hydrolysis. EPS was isolated from 10 ml of fermentation broth after the removal of cells by centrifugation and the addition of 2 volumes of chilled 95% ethanol to the supernatant. After incubation for 3 h

FIG. 1. Effect of fructose and sucrose concentration (in grams per liter) on the growth rate of *L. sanfranciscensis* LTH2590. Representative data of experiments performed in duplicate are shown.

at 4°C, the precipitate was collected by centrifugation (8,000 × g, 10 min). EPS was dissolved in deionized water and centrifuged again, and the supernatant was lyophilized. The lyophilized EPS was weighed, and the amount was indicated in grams liter⁻¹. For hydrolysis of EPS, 5% (vol/vol) perchloric acid (70% wt/vol) were added to the culture supernatants and incubated at 80°C for 2 h.

Characterization of oligosaccharide. Cells were removed from fermentation broth by centrifugation, and the oligosaccharide was separated from the supernatant by using a polyspher CH PB column (Merck). The molecular weight of the oligosaccharide was determined by gel permeation chromatography (Superdex peptide; Amersham Pharmacia Biotech, Uppsala, Sweden). Bidest. water was used as the mobile phase, and the determination was carried out at room temperature. Sucrose, raffinose, and stachyose were used for the calibration of the column. For the determination of the composition, the oligosaccharide purified by sequential runs on polyspher CH PB and Superdex peptide was hydrolyzed with 5% perchloric acid (2 h at 80°C). The monosaccharide concentrations were determined by HPLC. Information about the type of the monosaccharide linkages was obtained by the treatment of the purified oligosaccharide with β-fructosidase (yeast invertase; r-biopharm, Darmstadt, Germany) and subsequent analysis of the monosaccharides liberated by HPLC.

RESULTS

Effect of fructose on the utilization of sucrose. The EPS production from sucrose by *L. sanfranciscensis* LTH2590 was previously observed in media containing autoclaved sucrose where sucrose was partially hydrolyzed (19). To evaluate the ability of *L. sanfranciscensis* LTH2590 to metabolize sucrose in the absence of monosaccharides (fructosyltransferase acceptors), maximum growth rates were determined in media containing various amounts of sucrose and fructose sterilized by filtration. No growth was observed in the absence of fructose at sucrose concentrations ranging from 0 to 133 g liter⁻¹ (Fig. 1). The addition of 4.7 g of fructose liter⁻¹ resulted in a maximum growth rate of 0.49 h⁻¹ at a sucrose concentration of 2.3 g liter⁻¹, indicating that fructose (and possibly other monosaccharides) is essential for sucrose utilization by *L. sanfranciscensis* LTH2590. The further increase in fructose concentration had no effect on the maximum growth rate.

Kinetics of sucrose metabolism and EPS production in Su-

FIG. 2. Kinetics of microbial growth, EPS production, and fermentation products of *L. sanfranciscensis* LTH2590 in Su-MRS containing 60 g of sucrose liter⁻¹ and 5 g of fructose liter⁻¹ at pH 5.6. Δ , optical density at 578 nm (OD_{578}); $\blacktriangle$, EPS; $\bullet$, sucrose; $\circ$, mannitol; $\square$, fructose; $\blacksquare$, glucose; $\blacklozenge$, lactate; $\blacktriangledown$, ethanol; $\diamond$, acetate.

MRS. The kinetics of sucrose metabolism and EPS production by *L. sanfranciscensis* LTH2590 as well as the end products were determined for all fermentations at sucrose concentrations ranging from 20 to 160 g liter⁻¹ and at fructose concentrations of 5 or 15 g liter⁻¹. Figure 2 shows, as an example, the kinetics with 60 g of sucrose liter⁻¹ and 5 g of fructose liter⁻¹. Cells grew to 10^9 CFU ml⁻¹ within 20 h. The EPS production was coupled to the growth of the cells, and about 10 g of EPS liter⁻¹ was produced. The end products of sucrose metabolism were EPS, mannitol, lactate, and acetate or ethanol and the carbon recovery was 93%.

The amount of mannitol produced was higher than the amount of free fructose available at the beginning of the fermentation, demonstrating the ability of *L. sanfranciscensis* LTH2590 to hydrolyze sucrose into glucose and fructose. The hydrolysis of sucrose to glucose and fructose by *L. sanfranciscensis* LTH2590 was observed only when the free fructose was

FIG. 3. Effect of free fructose amount on the hydrolase activation by *L. sanfranciscensis* LTH2590 in fermentations with 60 g of sucrose liter⁻¹ and 15 g of fructose liter⁻¹ (A) or with 90 g of sucrose liter⁻¹ and 5 g of fructose liter⁻¹ (B). The amounts of consumed free fructose ($\blacktriangle$) and mannitol ($\circ$) and the sum of mannitol and free fructose ($\bullet$) are shown.

almost consumed (Fig. 3). The concentration of free fructose consumed and that of mannitol produced were identical as long as free fructose was available, indicating that the mannitol produced originated from free fructose only and no fructose was liberated from sucrose. The sum of free fructose and mannitol produced increased only after the free fructose was consumed, indicating that fructose was generated from the sucrose only when free fructose was almost completely consumed. The activation of sucrose hydrolysis by *L. sanfranciscensis* LTH2590 upon fructose depletion was observed in fermentations with 5 and 15 g of fructose liter⁻¹ and at all sucrose concentrations tested in this work (data not shown).

Effect of sucrose concentration on EPS production. The yield of EPS and end products formed by *L. sanfranciscensis* LTH2590 upon fermentations with various sucrose concentrations are shown in Table 1. The amount of sucrose consumed increased from 68 mmol liter⁻¹ at a sucrose concentration of 20 g liter⁻¹ to 245 mmol liter⁻¹ at a sucrose concentration of 160 g liter⁻¹. The increase in lactate production was not proportional to the sucrose consumption. The amounts of mannitol produced ranged from 98 to 111 mmol liter⁻¹ in fermentations with 15 g of fructose liter⁻¹ and 20 to 160 g of sucrose liter⁻¹, and in fermentations with 5 g of fructose liter⁻¹, mannitol concentrations ranged from 38 to 49 mmol liter⁻¹. Ace-

TABLE 1. Concentrations of substrates and end products of fermentations by *L. sanfranciscensis* in media with various sucrose and fructose contents

Substrate or end product (unit)	Result with fructose concn (g/liter) of:								
	15						5		
Sucrose (g liter ⁻¹)	20 ^a	30	60	70 ^a	120 ^a	160 ^a	20 ^a	60	90 ^a
Measured sucrose at <i>t</i> = 0 (mmol liter ⁻¹)	69	92	170	214	356	481	56	170	263
Consumed sucrose at <i>t</i> = end (mmol liter ⁻¹)	-68 ^b	-91	-109	-112	-160	-245	-58	-105	-141
Lactate (mmol liter ⁻¹)	74	86	73	97	103	137	62	98	67
Acetate (mmol liter ⁻¹)	50	55	57	56	52	62	25	25	24
Mannitol (mmol liter ⁻¹)	98	107	111	100	99	104	38	43	49
Fructose (mmol liter ⁻¹)	-75 ^b	-86	-73	-72	-79	-81	-22	-22	-30
Glucose (mmol liter ⁻¹)	0	0	12	6	13	6	0	0	0
Ethanol (mmol liter ⁻¹)	29	25	14	39	40	65	41	66	38
Glucose recovery ^c (%)	108	95	78	87	73	58	104	93	48

^a Values in these fermentations are representative of two independent experiments; the coefficient of variation between the two fermentations was generally in the range of 5 to 12%.

^b Negative values indicate consumption; positive values indicate production of metabolites.

^c Recovery of the glucose moiety from sucrose consumed in the end products: glucose, lactate, and acetate or ethanol.

tate production was independent of the sucrose concentration, and more acetate was produced in fermentations containing 15 g of fructose liter⁻¹. Glucose accumulation in the medium was only observed in fermentations with 15 g of fructose liter⁻¹ and at sucrose concentrations of 60 g liter⁻¹ or higher.

The glucose recoveries (amount of lactate produced per amount of glucose consumed) ranged from 95 to 108% in media containing up to 30 g of sucrose liter⁻¹ (Table 1). Taking into account the experimental error, recoveries in the range of 90 to 110% can be considered quantitative. In fermentations with sucrose concentrations greater than 60 g liter⁻¹, the glucose recoveries were lower. In medium containing 160 g of sucrose liter⁻¹, the carbon recovery was only 58%. The glucose recoveries decreased with increasing sucrose concentrations despite the fact that the EPS isolated from fermentation at any sucrose concentration consisted exclusively of fructose. The low glucose recovery observed at high sucrose concentrations correlated well with the peak area of an unknown metabolite produced during the sucrose fermentation (Fig. 4). The acid hydrolysis of the supernatant upon fermentation resulted in glucose and fructose recoveries of more than 92% (data not shown) and in the disappearance of the oligosaccharide peak in an HPLC chromatogram (Fig. 4A). The retention time of the oligosaccharide peak produced by *L. sanfranciscensis* was the same as that of 1-kestose (15 min) with polyspher CH PB, and the treatment of the sample with β -fructosidase or acid hydrolysis resulted in complete degradation of the oligosaccharide (Fig. 4A and data not shown). The same result was observed by using a second chromatography method, an anion-exchange chromatography with integrated amperometric detection (Fig. 4B). The oligosaccharide peak was collected from the polyspher CH PB column and subjected to gel permeation chromatography; the substance had an apparent relative molecular weight of 597 ± 27 . The hydrolysis of this oligosaccharide from supernatants of fermentations with various sucrose concentrations ($n = 3$) revealed a glucose-to-fructose ratio of 1 ± 0.3 to 2.2 ± 0.3 . The molecular weight and glucose-to-fructose ratio indicate that the oligosaccharide is a trisaccharide. The comparison of the retention time of the oligosaccharide and that of 1-kestose by two different chromatography methods revealed that the oligosaccharide or the

FIG. 4. Chromatographic separation of the culture supernatant of *L. sanfranciscensis* LTH2590 grown to stationary phase in Su-MRS (90 g liter⁻¹) by using a polyspher CH PB column coupled to a refractive index detector (A) and anion-exchange chromatography coupled to an electrochemical detector (B). The peak identification was based on external standards; shown in the figure are chromatograms of the 1-kestose external standards. In panel A, the chromatogram of the culture supernatant is additionally compared to that of the same culture supernatant upon acid hydrolysis. Chromatograms are offset by 250 mV.

FIG. 5. Effect of sucrose concentration on the amounts of EPS (A), mannitol (B), and kestose (C) produced by *L. sanfranciscensis* during fermentation in Su-MRS at a constant pH of 5.6. (A) Amounts of EPS produced by *L. sanfranciscensis* in Su-MRS containing various amounts of sucrose and 5 (▲) or 15 (Δ) g of fructose liter⁻¹. (B) Amounts of mannitol produced from sucrose by *L. sanfranciscensis* in Su-MRS containing various amounts of sucrose and 5 (●) or 15 (○) g of fructose liter⁻¹ and sucrose hydrolysis relative to total sucrose consumed in Su-MRS containing 5 (▲) or 15 (Δ) g of fructose liter⁻¹. (C) Amounts of 1-kestose produced by *L. sanfranciscensis* in Su-MRS containing various amounts of sucrose and 5 (▲) or 15 (Δ) g of fructose liter⁻¹.

major oligosaccharide produced by *L. sanfranciscensis* LTH2590 is 1-kestose.

The amount of EPS produced increased roughly in proportion to the sucrose concentration in the medium, and the fructose concentration showed no significant effect on the EPS production (Fig. 5). About 40 g of EPS liter⁻¹ was produced in medium containing 160 g of sucrose liter⁻¹ (Fig. 5A). The amount of mannitol produced from sucrose was virtually independent of fructose and sucrose concentrations in all fermentations (24 ± 6 mmol liter⁻¹, $n = 15$), demonstrating that the absolute hydrolase activity is independent of the sucrose concentration (Fig. 5B). However, more sucrose was consumed at higher sucrose concentrations in the medium (Table 1). This

means that the ratio of the mannitol produced from sucrose to consumed sucrose (relative hydrolase activity) decreased with increasing sucrose concentrations (Fig. 5B). Almost 40% of the fructose from the sucrose consumed was reduced to mannitol at a sucrose concentration of 20 g liter⁻¹, whereas at a sucrose concentration of 160 g liter⁻¹, only 10% of the fructose from the sucrose consumed was reduced to mannitol (Fig. 5B). The kestose production increased with increasing sucrose concentrations in the medium, and no significant effect of fructose concentration on the production of kestose could be observed (Fig. 5C).

DISCUSSION

The metabolism of sucrose by *L. sanfranciscensis* LTH2590 was only observed in media containing fructose which was apparently used as acceptor for the fructosyl residues. This activating mechanism of acceptors is commonly found for fructosyltransferases (25). Korakli et al. (19) suggested that levansucrase is the enzyme responsible for the EPS production by *L. sanfranciscensis*. We have recently demonstrated by PCR targeting the amino acid sequences of known levansucrases and by sequencing of the PCR product that the strain *L. sanfranciscensis* LTH2590 carries and expresses a levansucrase gene (29). Levansucrase (EC 2.4.1.10) is a fructosyltransferase enzyme that catalyses the following reaction:

In addition to the fructosyltransferase activity, levansucrases exhibit β -D-fructofuranosidase activity (25). Levansucrase has been recently isolated and purified from *L. reuteri* that produces polyfructan (32, 33). By increasing the initial sucrose concentration in the medium, more EPS was produced by *L. sanfranciscensis* LTH2590, whereas the amount of mannitol formed from sucrose in fermentations with sucrose contents ranging from 20 to 160 g liter⁻¹ indicated almost constant hydrolase activity. Thus, the relative hydrolase activity decreased by increasing sucrose concentrations. This conforms to the results obtained from a *Bacillus subtilis* levansucrase (9) and an *L. reuteri* fructosyltransferase (35), where increasing sucrose concentrations resulted in a shift of the enzyme activity from hydrolysis to fructosyltransferase. The highest fructosyltransferase activity by *B. subtilis* levansucrase was 32% at 37°C and 616 g of sucrose liter⁻¹ (9), whereas more than 90% of the fructose moiety from the sucrose were transformed by *L. sanfranciscensis* to oligo- or polysaccharides at sucrose concentrations higher than 120 g liter⁻¹.

The glucose recoveries were 95 to 108% at sucrose concentrations of up to 30 g liter⁻¹. A further increase in the sucrose concentration resulted in a decrease of the glucose recoveries. This was attributed to the production of a FOS characterized as 1-kestose. The amount of kestose produced increased with an increasing sucrose concentration in the medium. Crittenden and Doelle (4) reported that the percentage of fructose diverted to oligosaccharide by levansucrase from *Zymomonas mobilis* increased from 18% at a sucrose concentration of 92 g liter⁻¹ to 51% at a sucrose concentration of 280 g liter⁻¹.

Levansucrase isolated from various organisms were generally found to produce FOS. Hestrin et al. (15) reported first the production of FOS by *Aerobacter levanicum* levansucrase, and

FIG. 6. Sucrose metabolism by *L. sanfranciscensis*. FTF, fructosyltransferase.

Feingold et al. (10) have characterized this oligosaccharide as 1-kestose. *B. subtilis* levansucrase was also shown to produce 1-kestose (9). Structural analysis of the FOS produced by *Z. mobilis* levansucrase using ¹³C-nuclear magnetic resonance spectroscopy revealed that 98% of the total oligosaccharides were identified as 1-kestose and 1.5% were identified as nystose (3). The oligosaccharides produced by *Acetobacter diazotrophicus* had glucose-to-fructose ratios of 1:2 and 1:3 and were identified as 1-kestose and nystose, respectively (28). More recently, the formation of FOS by *L. reuteri* was reported (34). These oligosaccharides were characterized by anion-exchange chromatography, cation-exchange chromatography, and methylation analysis as 1-kestose and nystose, and 1-kestose was the major oligosaccharide produced from sucrose. This strain of *L. reuteri* carries two fructosyltransferase genes. Studies at the transcriptional and protein levels as well as the determination of enzyme activities of the wild-type strain and the structural elucidation of the polymer produced revealed that only one of these two genes, a levansucrase, is expressed in the host (31, 33, 34).

These results show similarities to the results of this study. The FOS produced by *L. sanfranciscensis* LTH2590 had a glucose-to-fructose ratio of 1:2. The retention time of the FOS from *L. sanfranciscensis* was identical with that of pure 1-kestose by two different chromatography methods, and the treatment of these purified oligosaccharides with β -fructosidase (yeast invertase) led to a complete hydrolysis to fructose and glucose, indicating that the linkage of the fructose is $\beta(2\rightarrow1)$ and that the oligosaccharide produced is 1-kestose. Low concentrations of other oligosaccharides produced by *L. sanfranciscensis* LTH2590 may have escaped our analytical setup. The enzymatic hydrolysis of 1-kestose by the action of β -fructosi-

dase was also observed by kestose produced by *A. levanicum* levansucrase (10) and *Z. mobilis* levansucrase (3). It is surprising that levansucrases which catalyze $\beta(2\rightarrow6)$ -linked polymers are also able to catalyze $\beta(2\rightarrow1)$ -linked oligosaccharides. Euzenat et al. (9) suggested that levansucrase produces 1-kestose but cannot transfer additional fructosyl residues onto it, leading to 1-kestose accumulation, while 6-kestose produced by levansucrase is rapidly used as acceptor for the production of levan. Since sucrose is a common part of dough formulas, and the sucrose concentration in the aqueous phase of doughs is high enough to expect kestose production, the use of kestose-producing lactobacilli in sourdough fermentation is an important step towards development of cereal-based functional foods.

In conclusion, it was shown that sucrose is metabolized by *L. sanfranciscensis* by the action of a fructosyltransferase enzyme, levansucrase, into glucose, kestose, and an EPS composed of fructose (Fig. 6). The glucose liberated is used as an energy source and degraded via a pentose-phosphate shunt. Sucrose is also hydrolyzed into glucose and fructose, and the latter is used as an electron acceptor and reduced to mannitol. The relative quantity of the alternative end products of sucrose medium are strongly dependent on the sucrose concentration.

ACKNOWLEDGMENTS

We thank Stephan Pröpsting for expert microbiological assistance. This work was supported by Deutsche Forschungsgemeinschaft grant no. F358/2.

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