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ARTICLE

Exopolysaccharide-Forming *Weissella* Strains as Starter Cultures for Sorghum and Wheat Sourdoughs

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The addition of sourdough fermented with lactic acid bacteria synthesizing organic acids and oligo- and exopolysaccharides (EPS) from sucrose enhances texture, nutritional value, shelf life, and machinability of wheat, rye, and gluten-free bread. This study compared acetate, mannitol, and oligosaccharide formation of EPS-producing strains of *Weissella* and *Leuconostoc* spp. to the traditional sourdough starter *Lactobacillus sanfranciscensis*. In broth, *Leuconostoc* strains generally formed acetate and mannitol, whereas *Weissella* produced only small amounts of acetate and no mannitol in the presence of sucrose. In the presence of sucrose and maltose, *Weissella* and *Leuconostoc* strains synthesized glucooligosaccharides and EPS. Strains of *Weissella* were employed as starter cultures for wheat and sorghum sourdough and formed 0.8−8 g kg^{-1} EPS and gluco-oligosaccharides but only low amounts of acetate and mannitol. In contrast, the formation of EPS from sucrose led to the production of high amounts of acetate and mannitol by *L. sanfranciscensis* LTH 2950 in wheat sourdough. This study indicates that *Weissella* strains are suitable starter cultures for wheat and sorghum sourdoughs and efficiently produce gluco-oligosaccharides and EPS.

KEYWORDS: *Weissella*; mannitol; EPS; oligosaccharides; gluten-free

INTRODUCTION

The use of sourdough improves the quality and increases the shelf life of bread. Sourdough delays staling, enhances the flavor, and increases the volume of wheat and rye breads (*1*). In rye baking, sourdough additionally solubilizes water-binding pentosans and inactivates amylases. The positive effects are associated with metabolites produced by lactic acid bacteria (LAB) during sourdough fermentation, including organic acids, exopolysaccharides (EPSs), and enzymes. Links between specific metabolic activities of sourdough cultures and product quality are well-described for traditional wheat and rye breads, but only limited data are available for gluten-free products (*2*). Gluten-free bread is characterized by decreased loaf volume, early staling, and a lower content of dietary fiber as compared to conventional wheat and rye products (*1*). Hydrocolloids are currently used to substitute gluten and to obtain gluten-free bread with acceptable sensory properties. EPSs formed by LAB from sucrose during sourdough fermentation have the potential to replace the use of hydrocolloids as additives. LAB produce a large structural variety of EPS; however, to date, only homopolysaccharides were shown to be useful in baking applications (*3*). Homopolysaccharides are glucan or fructan polymers composed of either glucose or fructose units, respectively. Glucans or fructans are synthesized from sucrose by extracellular glucan- or fructansucrases, respectively (*4*). In addition to EPS, glucan- and fructansucrases synthesize oligosaccharides by transferring glucose or fructose moieties, respectively, to suitable acceptor carbohydrates (*5*−*10*). The formation of oligosaccharides in sourdough depends on the presence of acceptor sugars (*5*, *11*). EPS formation has been frequently documented for key representatives of wheat and rye sourdough microbiota, including *Lactobacillus sanfranciscensis*, *Lactobacillus reuteri*, *Lactobacillus panis*, *Lactobacillus pontis*, and *Lactobacillus frumenti* (*12*). Strains of *Leuconostoc* and *Weissella* species that synthesize large amounts of dextran were isolated from Belgian, Italian, Greek, and French sourdoughs (*13*−*16*). Dextrans formed by glucansucrases (dextransucrases) of strains of *Leuconostoc* and *Weissella* are of high structural variety (*16*). Dextran produced during wheat sourdough fermentation increased loaf volume and improved crumb softness as compared to sourdough bread without dextran (*11*, *17*, *18*). Recently, improvement of gluten-free bread was achieved when *Weissella cibaria*-fermented sorghum sourdough was used (*5*).

The addition of sucrose not only enables EPS formation but also affects organic acid formation. In obligate heterofermentative LAB, glucose released from sucrose is metabolized to lactate, ethanol, and CO_2. In *L. sanfranciscensis* and *L. reuteri*, fructose is preferably used as an electron acceptor by the activity of a mannitol dehydrogenase, which results in reduced ethanol formation and formation of mannitol and acetate in a molar ratio of 2:1 (*19*). Strains of *Leuconostoc* produce lactate, acetate, ethanol, and CO_2 from fructose and form mannitol by the activity of a mannitol dehydrogenase (*20*, *21*). Higher levels of acetic acid in sourdough negatively affect LAB growth, EPS production, and bread volume (*13*, *22*).

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Table 1. Strains Utilized in This Study

Strains	origin	EPS from sucrose	identified GTF
L. geldium FUA3060[a]	meat	+[b]	ND
L. lactis FUA3310	feces	+	ND
L. carnosum ATCC49361	chilled stored meat	+	ND
L. mesenteroides FUA3090	kvass	dextran (*25*)	*dexLc* (*25*)
L. mesenteroides FUA3182	Finish vilii	+	ND
L. mesenteroides FUA3184	Finish vilii	+	ND
L. mesenteroides FUA3188	Finish vilii	+	ND
L. mesenteroides FUA3189	Danish ymer	+	ND
L. mesenteroides FUA3193	Danish ørsesundfil	+	ND
L. mesenteroides FUA3218	kimchi	+	ND
L. mesenteroides FUA3219	kimchi	+	ND
W. confusa ATCC10881		+	ND
W. minor ATCC35412	milking machine slime	+	ND
W. cibaria 2A	sourdough	glucan	ND
W. cibaria W58	sourdough	glucan	*dexWc*
W. cibaria 10M	sourdough	dextran (*5*)	*dexWc*
W. cibaria MG1	sourdough	dextran	ND
W. cibaria MG7	sourdough	dextran	ND
W. kimchi F33	sourdough	dextran	*dexWc*
W. kimchi F28	sourdough	dextran	*dexWc*
L. sanfransiscensis LTH2590	sourdough	levan (*42*)	*lev* (*42*)
L. sanfranciscensis LTH2581	sourdough	−	−
L. sanfranciscensis DSM20451	sourdough	fructan (*12*)	*lev* (*12*)

[a] Strains were obtained from the food microbiology culture collection of the University of Alberta (FUA). [b] Slimy colony morphology after growth on MRS agar plates supplied with 100 g L^{-1} sucrose. +, positive; −, negative; and ND, not detected.

Sucrose metabolism and mannitol and acetate formation have not been extensively investigated in EPS-forming strains of *Weissella*. Nevertheless, the ability of *Weissella* strains to synthesize substantial amounts of EPS makes them a suitable candidate to improve the quality of conventional and gluten-free bread. It was the aim of this study to screen potential EPS-forming *Weissella* strains for their ability to produce mannitol and acetate and to compare the metabolism of *Weissella* strains to EPS-forming strains of *Leuconostoc* and a traditional sourdough starter, *L. sanfranciscensis*, in buffer fermentations, sorghum, and wheat sourdough. *Weissella cibaria* and *Weissella kimchii* are phylogenetically closely related, and the reclassification of *W. kimchii* as *W. cibaria* has been proposed (*23*). Nevertheless, in this manuscript, the original strain identification was retained.

MATERIALS AND METHODS

Strains and Growth Conditions. Strains used in this study (**Table 1**) were grown in modified MRS (10 g of tryptone, 10 g of beef extract, 5 g of yeast extract, 2 g of triammonium citrate, 3 g of sodium acetate, 0.1 g of magnesium sulfate·$7H_2O$, 0.038 g of manganese sulfate·H_2O, 2 g of dipotassium sulfate, and 1 mL of Tween 80, per liter). GlcMRS and sucMRS were prepared with the addition of 110 and 85 mM glucose and sucrose, respectively. For the preparation of mMRS, 24 mM maltose, 22 mM glucose, and 22 mM fructose were added. The formation of oligosaccharides was tested in MRS supplied with 230 mM sucrose, 230 mM sucrose, and 55 mM maltose, 230 mM sucrose and 55 mM glucose, or 230 mM sucrose and 110 mM glucose. Working cultures were streaked on plates from glycerol stock stored at −80 °C. Single colonies were picked, subcultured once for 17 h and once for 14 h in glcMRS, inoculated at 1% in the final MRS, and incubated anaerobically at 30 °C for 16 h. All experiments were run in triplicate.

Wheat (Odlums, Dublin, Ireland) and sorghum (Twin Valley Mills, Nebraska) flour were used for the sourdough preparation using *W. kimchii* F28, *W. cibaria* MG1, or *L. sanfranciscensis* LTH2590 as starter cultures. Doughs were prepared with 40 g of flour, 40 mL of sterile tap water, and 6 g of sucrose. Strains were subcultured twice in mMRS media and inoculated to the doughs to obtain a concentration of 10^7 CFU g^{-1} sourdough. Control doughs were chemically acidified with 4 parts lactic acid (80%) and 1 part glacial acetic acid (100%) and to pH 4 and pH 3.6 for sorghum and wheat dough, respectively. Sourdoughs were incubated for 24 h at 30 °C. Cell counts and pH were determined at 4, 10, and 24 h; the persistence of starter cultures was confirmed by colony morphology and metabolite patterns.

Analysis of Metabolite and Oligosaccharide Formation in MRS and Doughs. Organic acids and mannitol were determined by HPLC with an Aminex HPX-87 column (Bio-Rad, Mississauga, Canada) at a temperature of 70 °C and a flow rate of 0.4 mL min^{-1} with 5 mM H_2SO_4 as the eluent. A refractive index detector was used for detection. The concentrations of mannitol, lactate, acetate, and ethanol were determined using external standards. Acetate present in MRS was subtracted from the amount synthesized by the strains. For sample preparations, equal volumes of perchloric acid were added to supernatants of fermentation liquor or sourdough to a final concentration of 3.7%, and samples were incubated at 4 °C overnight. Precipitates were removed by centrifugation (10000*g*, 10 min, 4 °C).

Sugars were analyzed with a CarbopacPA20 column (Dionex, Oakville, Canada) using water (A), 200 mM NaOH (B), and 1 M Na-acetate (C) as solvents at a flow rate of 0.25 mL min^{-1} with the following gradient: 0 min 30.4% B, 1.3% C, 22 min 30.4% B, and 11.34% C followed by washing and regeneration. Sucrose, glucose, fructose, maltose, panose, isomaltose, and isomaltotriose were used as external standards (all obtained from Sigma, Oakville, Canada). Oligosaccharides formed in MRS were directly analyzed from culture supernatant. Oligosaccharides synthesized in dough were extracted with H_2O at 80 °C for 2 h.

Characterization of EPS Monosaccharide Composition and Structure. The EPS composition and structure of *W. cibaria* MG1, *W. cibaria* MG7, *W. kimchii* F28, and *W. kimchii* F33 were determined as described by Schwab et al. (*5*). Briefly, the monosaccharide composition was analyzed after acid hydrolysis of the EPS; the linkage type was determined by enzymatic hydrolysis using dextranase and amyloglucosidase (*5*).

Determination of EPS Formation in MRS and Sourdough. EPS was analyzed by size exclusion chromatography using a Superdex 200 Column (GE Healthcare, Baie d'Urfe, Canada). Water was used as a solvent at a flow rate of 0.4 mL min^{-1}. EPS was detected with a RI detector.

The amount of EPS formed in MRS and sourdoughs was calculated using purified EPS from the respective strains as standards. EPS produced in MRS was analyzed directly from the supernatant. EPS was extracted from dough as previously described by Tieking et al. (*12*). Briefly, EPS was precipitated form aqueous dough extracts with ethanol, dialyzed against distilled water, and lyophilized. Freeze-dried samples were dissolved in distilled water (2 mg mL^{-1}) and quantified as described above. High molecular weight polysaccharides detected in chemically acidified doughs were subtracted from EPS starter culture samples to ensure that only EPS formed during sourdough fermentation and not polysaccharides from the flour were determined.

DNA Isolation and Polymerase Chain Reaction (PCR) Amplification of Dextransucrases. DNA was isolated from overnight cultures grown in MRS using DNeasy Blood & Tissue kit according to the instructions of the manufacturer (Qiagen, Mississauga, Canada). Dextransucrases were amplified using degenerate primers DegFor and DegRev (*24*), *Leuconostoc* dextransucrase-specific primer (*25*), or primer designed based on the dextransucrase sequence of *W. cibaria* (*9*) WcDexFor (5′-GCATCTTTCAATACTTGAGG-3′) and WcDexRev (CATGACTTGTTGGCATAGC-3′). Amplifications were carried out in a GeneAmp PCR System 9700 (Applied Biosystems, Streetsville, Canada). The amplification program was 94 °C for 3 min; 32 cycles of 94 °C for 30 s, 56 °C for 1 min, and 72 °C for 1 min 30 s; and finally 72 °C for 7 min. Amplicons were sequence by MacrogenUSA.

Nucleotide Sequence Accession Numbers. Nucleotide sequences for the partially sequenced glucansucrases are available in the EMBL Nucleotide Sequence Database under accession numbers FN706437 (*W. kimchii* F33), FN706438 (*W. kimchii* F28), FN706439 (*W. cibaria* W58), and FN706440 (*W. cibaria* 10M).

Table 2. Fructose Utilization, Formation of Mannitol and Acetate in Modified MRS Containing 24 mM Maltose, 22 mM Fructose, and 22 mM Glucose or 80 mM Sucrose by Strains of *Leuconostoc* and *Weissella* spp. and *L. sanfransiscensis* during Anaerobic Growth at 30 °C for 16 h[a]

	carbohydrate source					
	maltose, fructose, and glucose			sucrose		
	metabolites (mM kg^{-1})					
strains	fructose utilization[b]	mannitol formation	acetate formation	fructose utilization	mannitol formation	acetate formation
L. gelidum FUA3060	−2.4 ± 0.4	0	2.6 ± 0	ND	44.7 ± 23	18.8 ± 2.4
L. lactis FUA3310	−17.9 ± 0.1	17.2 ± 2.2	7.1 ± 2.8	ND	60.2 ± 1.5	20.4 ± 1.7
L. carnosum ATCC49361	−22.0 ± 0	13.5 ± 0	8.2 ± 1.8	ND	0	3.4 ± 2.1
L. mesenteroides FUA3090	−20.5 ± 0.1	16.2 ± 0	14.3 ± 1.4	ND	61.4 ± 2.9	28.6 ± 5.2
L. mesenteroides FUA3182	−4.2 ± 1.0	0	5.5 ± 2.8	ND	38.4 ± 5.7	22.6 ± 2.6
L. mesenteroides FUA3184	−4.8 ± 0.8	0	4.1 ± 1.8	ND	37.1 ± 9.7	23 ± 7.2
L. mesenteroides FUA3188	−17.5 ± 1.4	14 ± 0.6	7.6 ± 7.2	ND	4.9 ± 2.5	11.9 ± 6.8
L. mesenteroides FUA3189	0	0	1.3 ± 1.5	ND	9 ± 5	10.8 ± 4
L. mesenteroides FUA3193	−19.2 ± 2.4	15.6 ± 0.8	14.1 ± 3.0	ND	29.4 ± 2.9	16.2 ± 0.9
L. mesenteroides FUA3218	−22 ± 0	18.5 ± 0.6	5.2 ± 0.2	ND	68.8 ± 2.4	27.8 ± 2.7
L. mesenteroides FUA3219	−4.2 ± 2.7	0	2.3 ± 5	ND	1.8 ± 3.2	5.9 ± 2.7
W. confusa ATCC10881	−22 ± 0	0	14 ± 0	ND	26.6 ± 4.9	25.0 ± 5.8
W. minor ATCC35912	−22 ± 0	11.9 ± 0.1	14.6 ± 5.2	ND	0	12.3 ± 0.9
W. cibaria 2A	−5.2 ± 1.5	0	5.0 ± 2.7	ND	0	8.2 ± 0.5
W. cibaria W58	−20.4 ± 2.3	0	0.3 ± 0.4	ND	0	4.9 ± 3.6
W. cibaria 10M	−4.8 ± 0.5	0	2.5 ± 0	ND	0	2.3 ± 3.0
W. cibaria MG1	.-4.9 ± 0.6	0	1.0 ± 0.3	ND	42.4 ± 3.3	21.7 ± 0.6
W. cibaria MG7	−5.7 ± 0.9	0	4.1 ± 3.5	ND	3.4 ± 6	12.0 ± 2.6
W. kimchi F33	−2.8 ± 0.5	0	1.6 ± 0.7	ND	0	−0.2 ± 1.1
W. kimchi F28	−2.1 ± 0.7	0	1.6 ± 0	ND	0	5.3 ± 1.1
L. sanfransiscensis LTH2581	−22 ± 0	22.1 ± 2.4	8.0 ± 0.4		NG	
L. sanfransicensis DSM20451	−20.4 ± 0.5	19.0 ± 1.8	8.6 ± 3.4		NG	

[a] ND, not determined; NG, no growth. [b] Negative values indicate the amount of fructose consumed.

RESULTS

Acetate and Mannitol Formation in MRS. Mannitol and acetate formation of *Leuconostoc* and *Weissella* spp. was strain- and substrate-dependent (**Table 2**). When grown in MRS with 24 mM maltose, 22 mM glucose, and 22 mM fructose, *L. lactis* FUA3310, *L. carnosum* ATCC49361, and three strains of *L. mesenteroides* utilized fructose and produced mannitol and acetate. With the exception of *Weissella minor* ATCC35912, none of the *Weissella* strains tested formed mannitol, and all synthesized only low amounts of acetate when grown with maltose, glucose, and fructose. *L. sanfranciscensis* LTH2581 and DSM20451 only grew in MRS supplied with maltose, glucose, and fructose, quantitatively reduced fructose to mannitol, and formed acetate in an approximate ratio of 2:1. In the presence of 85 mM sucrose, all *Leuconostoc* strains except *L. carnosum* ATCC49361 formed mannitol and acetate. With the exception of *W. cibaria* MG1 and MG7 and *W. confusa* ATCC10881, all *Weissella* strains formed small amounts of acetate (between 2 and 12 mM) but no mannitol when grown with sucrose. *W. cibaria* MG7 synthesized small amounts of mannitol, whereas *W. cibaria* MG1 and *W. confusa* ATCC10881 synthesized mannitol and acetate in a ratio of 2:1 or in equimolar amounts, respectively.

Sucrose Utilization, Oligosaccharide, and EPS Formation in MRS with Sucrose, Sucrose, and Maltose or Sucrose and Glucose. Strains of *Leuconostoc* and *Weissella* formed EPS in the presence of 230 mM sucrose. Gluco-oligosaccharides were formed in addition to EPS when the acceptor maltose was present (**Figure 1** and **Table 3**). Gluco-oligosaccharide formation was indicated by the presence of panose or higher oligosaccharides of the glucosylated panose series (*5*). Oligosaccharide formation was not observed when strains were incubated in the presence of 230 mM sucrose and 55 mM glucose (data not shown). Only trace amounts of oligosaccharides were synthesized when strains grew in MRS containing 230 mM sucrose and 110 mM glucose (data not shown). *L. mesenteroides* FUA3182, *L. mesenteroides* FUA3184, and *W. minor* ATCC35912, which did not synthesize panose or gluco-oligosaccharides, are potential fructan-forming strains.

Two distinctive patterns were observed in gluco-oligosaccharide formation with sucrose and maltose (**Figure 1**). In OS pattern 1, the short-chain gluco-oligosaccharides panose and panose with up to four glucosyl units were detected. In OS pattern 2, only small amounts of panose and glucosylated panose with a degree of polymerization of up to 14 were synthesized (**Figure 1**). Strains synthesizing OS pattern 2 also formed approximately twice as much EPS when grown with sucrose and maltose as compared to EPS formation in the presence of sucrose only (**Table 3**). Additionally, EPS produced with maltose and sucrose was of higher molecular weight (**Figure 2**). Strains producing no gluco-oligosaccharides formed similar amounts of EPS when grown in MRS supplied with sucrose and maltose or only sucrose (**Table 3**).

Growth and Metabolite Formation in Sourdoughs. Sorghum sourdough contained initial higher amounts of glucose and fructose (13 and 14 mM kg^{-1}) as compared to wheat (1.4 and 1.5 mM kg^{-1}), respectively (t = 0 h). In sorghum sourdough, glucose levels increased during the first 10 h and were constant until 24 h. In contrast, in wheat dough, a gradual increase of glucose throughout the fermentation was observed. The fructose concentration increased during sorghum sourdough fermentation but not during the fermentation of wheat dough. At the beginning of fermentation (t = 0 h), maltose was only present in wheat (17 mM kg^{-1}), whereas maltose was detected in sorghum only after 4 h of incubation. Maltose and the added sucrose were completely metabolized during fermentation in both substrates.

Wheat and sorghum flours were fermented with *W. kimchii* F28, *W. cibaria* MG1, or *L. sanfranciscensis* LTH2590. During 24 h of fermentation with *W. kimchii* F28 or *W. cibaria* MG1, the pH in sorghum sourdough and wheat sourdough decreased to 4.1

and 4.0 and 3.9 and 3.9, respectively. Cell counts of both *Weissella* strains were higher in sorghum sourdough (9.5 log CFU g^{-1}) than in wheat sourdough (8.9 CFU g^{-1}). *L. sanfranciscensis* only grew in wheat sourdough and reached 8.8 CFU g^{-1} after 10 h of incubation; after 24 h, the pH decreased to pH 3.5, and cell counts had dropped to 7.6 CFU g^{-1}.

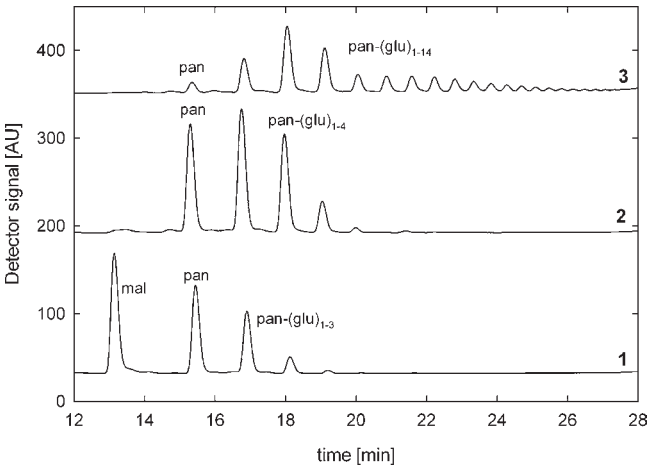

Figure 1. Formation of gluco-oligosaccharides in the presence of 230 mM sucrose and 55 mM maltose during fermentation in MRS at 30 °C for 16 h. Gluco-oligosaccharides of OS pattern 1 were produced by *L. mesenteroides* FUA3218 (1) and FUA3090 (2), and OS pattern 2 was synthesized by *W. cibaria* MG1 (3). Gluco-oligosaccharides were analyzed from culture supernatant using HPAEC-PAD. Abbreviations: mal, maltose; pan, panose; pan-$(glu)_n$, glucosylated panose; and *n*, degree of polymerization.

W. kimchii F28 and *W. cibaria* MG1 synthesized equimolar amounts of lactate and ethanol in wheat and sorghum sourdough (**Figure 3**). Generally, lactic acid and ethanol were synthesized in higher amounts in sorghum sourdoughs. In contrast to *L. sanfranciscensis* LTH2590, which formed 155 ± 30 mM kg^{-1} mannitol and 45 ± 3 mM kg^{-1} acetate during fermentation in wheat sourdough, no mannitol and only small amounts of acetate (below 6 mM kg^{-1} dough) were formed by *W. kimchii* F28 and *W. cibaria* MG1 in wheat. In sorghum sourdough, *W. kimchii* F28 and *W. cibaria* MG1 formed 10 and 8 mM kg^{-1} acetate after

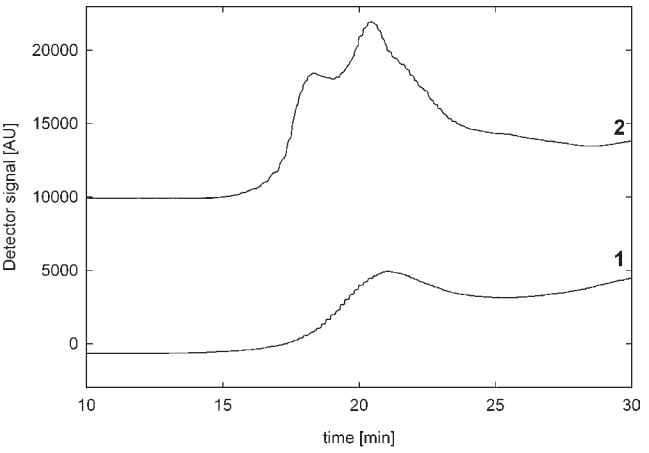

Figure 2. EPS synthesized by *W. cibaria* MG1 during growth in MRS supplied with 230 mM sucrose (1) or 230 mM sucrose and 55 mM maltose (2). EPS formation was analyzed by size exclusion chromatography.

Table 3. Utilization of Sucrose, Oligosaccharide (Panose), and EPS Formation of Strains of *Leuconostoc* and *Weissella* spp. during Growth in Modified MRS Containing 230 mM Sucrose or 230 mM Sucrose and 55 mM Maltose at 30 °C for 16 h

	substrate in mMRS				
	sucrose		sucrose/maltose		
	metabolites (sucrose and panose in mM kg^{-1} and EPS in g kg^{-1})				
strains	remaining sucrose	EPS formed	remaining sucrose	panose	EPS
			panose or no oligosaccharide		
L. carnosum ATCC49367	57.8 ± 17.2	2.4 ± 0.3	58.5 ± 7.1	2.7 ± 0.1	1.9 ± 0.2
L. mesenteroides FUA3182	53.7 ± 14.9	2.3 ± 0.5	76.8 ± 9.6	no oligosaccharide	1.9 ± 0.1
L. mesenteroides FUA3184	57.7 ± 16.6	2.2 ± 0.3	87.2 ± 3.2	no oligosaccharide	2.7 ± 0.1
L. mesenteroides FUA3193	79.4 ± 0.1	4.7 ± 0.1	81.4 ± 5.3	1.8 ± 1.3	3.5 ± 0.5
W. minor ATCC35912	4.3 ± 1.3	2.3 ± 0.1	74.2 ± 2.5	0	2.2 ± 0.2
			OS pattern 1		
L. lactis FUA3310	17.2 ± 12.5	4.9 ± 1.0	0	2.6 ± 1.0	2.7 ± 0.9
L. mesenteroides FUA3090	14.0 ± 9.4	21.1 ± 1	3.5 ± 3.0	7.4 ± 0.1	8.7 ± 0.2
L. mesenteroides FUA3218	38.4 ± 8.3	4.7 ± 0.1	37.9 ± 1.0	6.4 ± 0.1	3.5 ± 0.5
W. cibaria 2A	55.8 ± 7.0	4.7 ± 1.3	70.2 ± 3.5	1.6 ± 0.1	3.4 ± 0.4
W. cibaria W58	51.1 ± 3.8	2.4 ± 0.1	48.6 ± 13.4	2.4 ± 0.2	1.8 ± 0.2
			OS pattern 2		
L. gelidum FUA*3060*	68.2 ± 5.7	2.1 ± 0.2	0	1.2 ± 0.2	11.9 ± 0.5
L. mesenteroides FUA3188	68.2 ± 3.3	3.1 ± 0.7	35.4 ± 20.8	0.2 ± 0.3	6.4 ± 0.9
L. mesenteroides FUA3189	58.7 ± 14.1	3.4 ± 0.5	1.6 ± 1.5	0.6 ± 0.6	5.2 ± 1.9
L. mesenteroides FUA3219	14.6 ± 1.7	3.9 ± 1.8	4.2 ± 7.2	0.9 ± 0.3	3.8 ± 0.4
W. confusa ATCC10881	46.6 ± 24.5	2.7 ± 1.3	0.5 ± 0.8	0	7.7 ± 1
W. cibaria 10M	46.5 ± 2.2	7.5 ± 1.3	23.3 ± 5.6	0	14.1 ± 0.9
W. cibaria MG1	53.8 ± 17.0	3.0 ± 0.3	0	0	10.8 ± 0.8
W. cibaria MG7	48.9 ± 22.6	3.6 ± 0.8	8.8 ± 2.3	0	8.4 ± 0.7
W. kimchi F33	42.4 ± 9.6	3.1 ± 0.4	18.5 ± 0.8	0	8.9 ± 0.4
W. kimchi F28	41.5 ± 12.2	3.4 ± 0.3	18.9 ± 7.4	0	6.2 ± 2.6

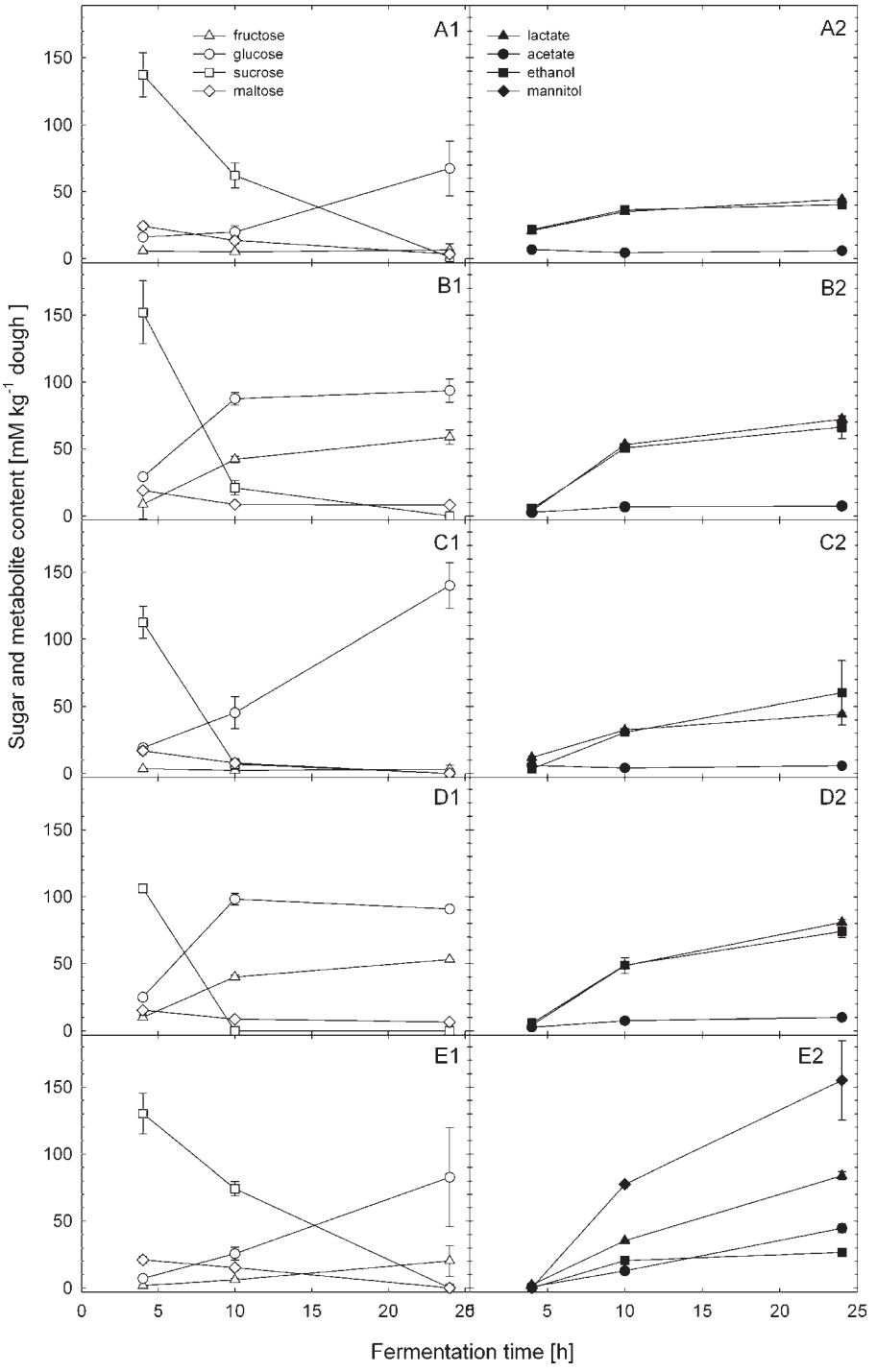

Figure 3. Glucose (○), fructose (△), maltose (◇), and sucrose (□) content (1) and lactate (▲), acetate (●), ethanol (■), and mannitol (♦) formation (2) during wheat (**A**, **C**, and **E**) and sorghum (**B** and **D**) sourdough fermentation by *W. kimchii* F28 (**A** and **B**), *W. cibaria* MG1 (**C** and **D**), and *L. sanfranciscensis* LTH2950 (**E**).

24 h of fermentation, respectively. Trace amounts of mannitol were detected in sorghum fermented with *W. cibaria* MG1.

Oligosaccharide and EPS Formation in Sourdough. *W. kimchii* F28 and *W. cibaria* MG1 formed oligosaccharides during growth in sorghum and wheat sourdough (**Figure 4**). Depending on the flour type, fermentation time, and strain used for the fermentation, different OS patterns were detected. In sorghum sourdough, both strains formed isomalto-oligosaccharides in 10 h that were still present after 24 h of fermentation. In wheat sourdough, gluco-oligosaccharides comprised of panose and glucosylated panose were synthesized. *W. kimchii* F28 formed gluco-oligosaccharides with 1 or 2 glucose units linked to panose after 4 and 10 h of fermentation. After 24 h, long-chain gluco-oligosaccharides consisting of panose linked with up to eight glucose units were detected. *W. cibaria* MG1 synthesized short-chain gluco-oligosaccharides during 4 h incubations. After 10 h, only a small amount of panose was still present, but gluco-oligosaccharides with a polymerization degree up to 13 were produced. After 24 h, only long-chain gluco-oligosaccharides were detected. *W. cibaria* MG1 produced higher amounts of EPS in both sourdoughs than *W. kimchii* F28. In sorghum and wheat sourdough, 8.0 ± 3.0 and 4.7 ± 0.4 g EPS kg^{-1} dough was formed

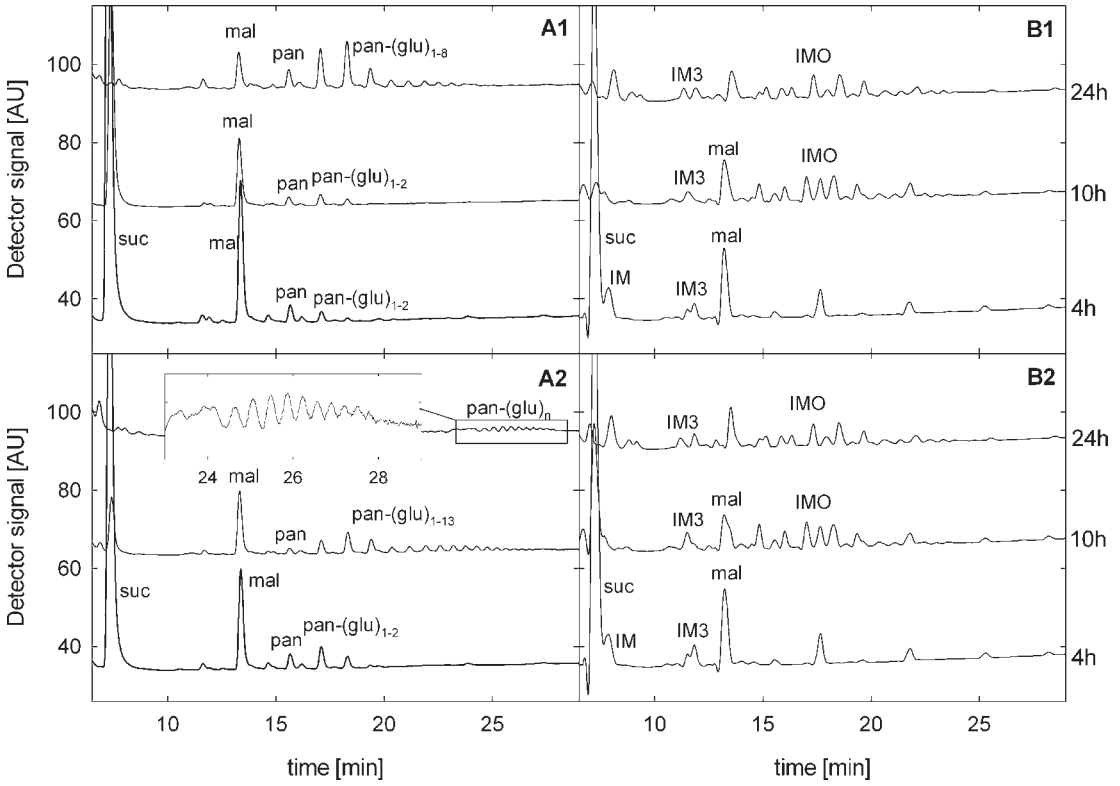

Figure 4. Formation of oligosaccharides in wheat (**A**) and sorghum (**B**) sourdough by *W. kimchi* F28 (1) and *W. cibaria* MG1 (2). Oligosaccharides were extracted with H_2O at 80 °C for 2 h and analyzed using HPAEC-PAD. Abbreviations: suc, sucrose; mal, maltose; pan, panose; pan-(glu)$_n$, glucosylated panose; *n*, degree of polymerization; IM, isomaltose; IM3, isomaltotriose; and IMO, isomaltooligosaccharides.

by *W. cibaria* MG1 as compared to 4.3 ± 1.9 and 0.8 ± 0.6 g EPS kg^{-1} dough formed by *W. kimchii* F28, respectively. Oligosaccharide formation was not observed in wheat sourdoughs fermented with *L. sanfranciscensis* LTH2590; however, 2.2 ± 1.1 g EPS kg^{-1} dough was produced by this strain.

PCR Amplification of Dextransucrase-Encoding Genes. The presence of dextransucrase genes was verified for *W. cibaria* 10M and W58 and for *W. kimchii* F28 and F38. Amplicons obtained by PCR from *W. cibaria* W58 and 10M and *W. kimchii* F28 and F33 genomes using *Weissella* dextransucrase-specific primers WcDexFor and WcDexRev showed the highest homology to a *L. fermentum* (*9*) glucansucrase and a *W. cibaria* dextransucrase (*26*) (*W. cibaria* W58 and 10M 98 and 97% homologous in 327 AA, *W. kimchii* F28 100 and 98% homologous in 324 AA, *W. kimchii* F33 100 and 98% homologous in 209 AA to *L. fermentum* glucansucrase, and *W. cibaria* dextransucrase, respectively.) No further amplicons were obtained using dextransucrase-specific primers WcDexFor and WcDexRev, degenerate primers DegFor and DegRev (*24*), or *Leuconostoc* dextransucrase-specific primer (*25*).

DISCUSSION

LAB are the dominant microorganisms in sourdoughs, and dough rheology, and the flavor and nutritional properties of sourdough bread greatly depend on the metabolic activity of LAB. Traditional wheat and rye sourdough fermentations are characterized by a highly adapted microbiota of LAB and yeast (*27*, *28*). Fermentation parameters and dough yield are recognized as important determinants of the microbiota composition; however, the impact of the raw material on the fermentation microbiota remains a matter of discussion (*29*−*31*). The obligate heterofermentative *L. sanfranciscensis* or closely related organisms such as *Lactobacillus rossiae*, *Lactobacillus brevis*, and *Lactobacillus spicheri* are invariably present in wheat and rye sourdoughs and frequently occur in association with *Lactobacillus plantarum* and *Lactobacillus paraalimentarius*. Thermophilic lactobacilli such as *L. reuteri*, *L. fermentum*, or *L. pontis* become dominant when fermentation is carried out at a higher temperature. Sourdoughs prepared with the cereals of the genus *Triticale*, for example, wheat and rye, are populated by a comparable microbiota (*32*). However, the microbiota of sorghum sour fermentations, which are a staple food in Africa, differ from wheat and rye sourdough microbiota. *L. plantarum* and *L. fermentum* are frequently isolated as dominant strains of the fermentation microflora, which also contain often *Lactobacillus rhamnosus*, *L. brevis*, pediococci, and *Weissella* species (*33*−*35*). In this study, the traditional wheat and rye starter *L. sanfranciscensis* LTH 2590 failed to grow in sorghum sourdough, most likely due to the lack of maltose at the beginning of fermentation, which is the preferential carbohydrate source for this species (*36*). Maltose was not present in sorghum flour but was released only gradually during fermentation through hydrolysis of starch by the activity of the α-amylases and β-amylases. In contrast, high levels of glucose in sorghum sourdough due to the activity of the glucoamylases and the metabolism of sucrose favored the growth of *Weissella* species, since glucose is the preferable carbohydrate of *W. cibaria* (*37*). *W. cibaria* MG1 and *W. kimchii* F28 also reached high cell counts in wheat sourdoughs, confirming the potential to act as starter cultures in wheat and sorghum fermentations. In sorghum sourdoughs, both *Weissella* strains reached higher cell counts and formed increased amounts of lactic acid and ethanol as compared to in wheat sourdough. However, the final pH was comparable in both substrates, indicating an increased buffer capacity of the sorghum flour.

EPS formation has been investigated in detail in rye and wheat sourdough (*11*, *12*, *18*, *22*). The concentration of EPS

accumulated by *L. sanfranciscensis* and *L. reuteri* during fermentation is sufficient to exert desirable technological effects (*12*, *22*). Strains of *Weissella* synthesize high amounts of EPS in buffer and wheat fermentations (*7*−*9*), but only one study so far investigated EPS formation in sorghum fermentations (*5*). Monosaccharide analysis and structure analysis identified the EPSs synthesized by *W. cibaria* and *W. kimchii* as glucans (dextrans). The presence of dextransucrase-encoding genes could be confirmed in four *Weissella* strains using specific primers. Confirming earlier reports by Bounaix et al. (*16*), DegFor and DegRev (*24*) failed to amplify dextransucrase-encoding genes in EPS-synthesizing *Weissella* strains. The amounts of EPS synthesized by *W. cibaria* MG1 and *W. kimchii* F28 in wheat and sorghum sourdoughs were comparable to levels formed by *W. confusa* in wheat sourdough (*11*) and higher than reported for *W. cibaria* WC4 and *W. cibaria* 10M in wheat and sorghum fermentations, respectively (*5*, *18*). Hydrocolloids are effective as dough improvers when added at a level of 0.3% (*38*), and EPS formation to levels ranging from 0.9 to 1.6% shows the potential of *Weissella* strains to substitute addition of hydrocolloids.

In addition to EPS, most *Weissella* and *Leuconostoc* strains tested in this study formed gluco-oligosaccharides in the presence of the acceptor sugar maltose. In buffer fermentations, strain-dependent correlations between the presence of acceptors, oligosaccharide, and EPS formation were observed. The presence of maltose either generally increased EPS yield and molecular weight of EPS (OS pattern 2) or led to a reduction of EPS synthesis but did not affect EPS size (OS pattern 1). The latter is in agreement with observations made by Rodrigues et al. (*39*), which reported that maltose decreased EPS yield formed by *L. mesenteroides* B512F but did not alter EPS size distribution.

W. kimchii F28 and *W. cibaria* MG1, which formed similar patterns of gluco-oligosaccharides and comparable amounts of EPS in buffer fermentations with sucrose and maltose, were further applied in wheat and sorghum fermentations. In sourdough fermentations, the presence of maltose and glucose as acceptor sugars affected oligosaccharide formation by *W. cibaria* MG1 and *W. kimchii* F28. Maltose was the immediately available carbohydrate source in wheat fermentations and therefore the preferential acceptor sugar. The kinetics of oligosaccharide synthesis in wheat was strain-dependent. *W. cibaria* MG1 rather elongated oligosaccharide during the course of fermentation, whereas *W. kimchii* F28 enriched panose and glycosylated panose up to a polymerization degree of 8.

In sorghum sourdough, glucose was the available acceptor carbohydrate. Because of a lack of maltose, a higher content of glucose at the beginning of fermentation, and the constant supply of glucose throughout the fermentation, *W. cibaria* MG1 and *W. kimchii* F28 produced isomaltose, isomaltotriose, and isomalto-oligosaccharides (*5*). In buffer fermentations, glucose was metabolized by the strains and not available as an acceptor to form oligosaccharides. Isomalto-oligosaccharide formation has also been reported in sorghum and wheat sourdoughs (*5*, *22*). Gluco-oligosaccharides are known to selectively stimulate the growth of bifidobacteria. In particular, long-chain gluco-oligosaccharides with a degree of polymerization of 3 or higher are preferred to short-chain oligosaccharides because of the longer persistence in the colon (*40*). Different from fructo-oligosaccharides, gluco-oligosaccharides are not digested by yeast during proofing and are still present in the final bread (*5*).

The formation of oligo- and EPSs from added sucrose results in the release of either glucose or fructose and thus affects organic acid formation. In buffer fermentations containing sucrose, strains of *Weissella* utilized fructose as a carbon source but did not synthesize mannitol and formed only small amounts of acetate with the exemption of *W. cibaria* MG1. In contrast, EPS-forming strains of *Leuconostoc* fermented fructose and utilized it as an electron acceptor, resulting in the formation of mannitol and acetate. In the presence of maltose and glucose, *L. sanfranciscensis* uses fructose as an electron acceptor through mannitol dehydrogenase activity (*39*, *41*). The failure of *L. sanfranciscensis* to grow with sucrose as a sole carbohydrate source confirms previous observations (*41*).

In sourdough fermentations, both *Weissella* strains and *L. sanfranciscensis* LTH2590 efficiently utilized sucrose. The formation of dextran by *Weissella* during sourdough fermentation led to a release of fructose. In sorghum sourdoughs fermented with strains of *Weissella*, fructose levels increased over 24 h of fermentation, whereas only a small amount of fructose was detected in wheat sourdoughs. The formation of small amounts of acetate in sorghum and wheat sourdoughs and the depletion of fructose in wheat sourdough indicates that *W. cibaria* MG1 and *W. kimchii* F28 metabolized fructose by the pentose−phosphate pathway. In contrast, the levan-producing strain *L. sanfranciscensis* LTH2590 transformed most of the fructose released from sucrose by the hydrolysis reaction catalyzed by its levansucrase into mannitol and synthesized up to 45 mM acetate in wheat fermentations (*42*). The production of acetate during baking is beneficial due to its antifungal properties, but high concentrations cause off-flavor and compromise crumb structure (*22*). A fermentation quotient (ratio of the amount of lactate and acetate) of 1.9 obtained with *L. sanfranciscensis* is well below a fermentation quotient of 4, postulated to be optimal for wheat bread (*43*).

In conclusion, sucrose addition in quantities to allow sufficient EPS production by *L. sanfranciscensis* and *L. reuteri* leads to acetate formation that negatively affects bread texture and flavor (*22*, *42*). Therefore, the application of EPS-forming *Weissella* strains synthesizing only low amounts of acetate and mannitol broadens the spectrum of EPS-forming starter cultures suitable for sorghum and wheat fermentations. Oligosaccharide formation by strains of *Weissella* depends on flour and the choice of strain.

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