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Lactose- and cellobiose-derived branched trisaccharides and a sucrose-containing trisaccharide produced by acceptor reactions of *Weissella confusa* dextransucrase

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ABSTRACT

Dextran-producing *Weissella* have received significant attention. However, except for maltose, the acceptor reactions of *Weissella* dextransucrases with different sugars have not been investigated. The action of recombinant *Weissella confusa* VTT E-90392 dextransucrase was tested with several potential acceptors, particularly, analogs lactose and cellobiose. The major acceptor products of both disaccharides were identified as branched trisaccharides, with a glucosyl residue α-(1→2)-linked to the acceptor's reducing end. An additional product, isomelezitose (6^{Fru}-α-Glc*p*-sucrose), was also produced when using lactose as an acceptor. This is the first report of the synthesis of isomelezitose by a dextransucrase. The NMR spectra of the three trisaccharides were fully assigned, and their structures were confirmed by selective enzymatic hydrolysis. The trisaccharides prepared from $^{13}C_6^{glc}$ sucrose and lactose were analyzed by ESI-MS^n, and the fragmentation patterns of these compounds were characterized.

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1. Introduction

Various lactic acid bacteria from genera *Leuconostoc*, *Lactobacillus*, *Streptococcus*, and *Weissella* produce extracellular α-glucans to protect them from environmental stress (Leemhuis et al., 2013). Glucansucrases catalyze the synthesis of α-glucans from glucopyranosyl residues of sucrose, and fructose is released. In the presence of suitable hydroxyl-group containing acceptors, such as low-molecular weight carbohydrates, glucansucrases also catalyze so-called acceptor reactions, where the glucopyranosyl unit from sucrose is transferred onto an acceptor other than the growing glucan chain, forming glucooligosaccharides, in addition to α-glucan polymers (Leemhuis et al., 2013). The high selectivity and regiospecificity of glucansucrases compared to chemical approaches make them useful synthetic tools for high-value carbohydrate-based molecules (André, Potocki-Véronèse, Morel, Monsan, & Remaud-Siméon, 2010). Additional advantages are their relaxed and broad substrate specificity toward many acceptors and the low cost of the donor substrate sucrose (André et al., 2010).

Based on the structure of the glucan they synthesize, glucansucrases are classified into dextransucrases, mutansucrases, reuteransucrases, and alternansucrases (Leemhuis et al., 2013). Dextransucrases (EC 2.4.1.5), for example, synthesize dextrans, which contain mainly consecutive α-(1→6) linkages and α-(1→2), α-(1→3), or α-(1→4) branch linkages (Maina, Virkki, Pynnönen, Maaheimo, & Tenkanen, 2011). The degree of branching varies according to the origin of the dextransucrase. Glucansucrases belong to the glycoside hydrolase (GH) family 70 based on amino acid sequence similarity and structure analogy. They are structurally and mechanistically related to GH13 and GH77 enzymes (Leemhuis et al., 2013). The three-dimensional structure of truncated glucansucrases revealed that they exhibit a U-type shape, organized into five domains. The catalytic domain adopts a $(\beta/\alpha)_8$ barrel fold and harbors a catalytic triad, which is composed of two aspartates and one glutamate (Leemhuis et al., 2013). The catalysis of glucansucrases is proposed to be a two-step process, starting with the cleavage of the sucrose substrate and followed by the formation of a covalent β-glucosyl-enzyme intermediate. In the second step, the glucosyl moiety is transferred to an acceptor, with retention of the α-anomeric configuration. The type of glycosidic linkage that

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formed depends on the orientation in which the acceptor is guided toward the reaction center (Leemhuis et al., 2013).

Acceptor reactions of dextransucrases, especially those of *Leuconostoc* dextransucrases, have been exploited to synthesize glucooligosaccharides for use in food and health applications (Chung & Day, 2002; Goffin et al., 2011; Kothari & Goyal, 2014; Seo, Kim, Eom, & Han, 2007). The glucosidic linkage type formed in the product is dependent on the acceptor substrate and the enzyme specificity. Maltose is the most studied acceptor due to its high effectiveness. Its initial product, panose (α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→4)-D-Glc*p*), can be elongated by successive attachment of glucosyl units, primarily to 6-OH of the nonreducing end glucose, thus forming a series of isomaltooligosaccharides (IMOs). The IMOs produced by *Leuconostoc mesenteroides* NRRL B-1299 dextransucrase also contain single-unit α-(1→2) branches and are marketed for their prebiotic properties (Goffin et al., 2011). Among dextransucrases, only dextransucrase from *Lc. mesenteroides* NRRL B-512F has been intensively studied using acceptors other than maltose (Robyt, 1995; Robyt & Eklund, 1983). Lactose, lactulose, and cellobiose were demonstrated previously to form trisaccharide acceptor products with an α-(1→2)-linked glucosyl residue instead of an α-(1→6)-linked residue. These trisaccharides showed *in vitro* the ability to selectively stimulate the growth of probiotic bacteria (Argüello Morales, Remaud-Simeon, Willemot, Vignon, & Monsan, 2001; Díez-Municio, Herrero, Jimeno, Olano, & Moreno, 2012a; García-Cayuela et al., 2014; Ruiz-Matute et al., 2011).

Weissella spp. represent promising dextran producers, especially in sourdough bread. Unlike many *Leuconostoc* spp., they do not convert fructose into mannitol, with concomitant acetate production. Therefore, the technological benefits of dextran are not overridden by high acidity in sourdough bread baking (Galle, Schwab, Arendt, & Gänzle, 2010; Katina et al., 2009). The dextrans produced by *Weissella* spp. are structurally similar to dextran from *Lc. mesenteroides* B-512F, consisting predominantly of α-(1→6) linkages and only a few α-(1→3) branch linkages (Maina et al., 2011). Dextransucrases from *Weissella confusa* have only recently been characterized (Amari et al., 2012; Kajala et al., 2015; Shukla et al., 2014). Except for maltose, the acceptor reactions of *Weissella* dextransucrases have not been examined. The maltose reaction produced a typical homologous series of IMOs (Shukla et al., 2014). Previous research demonstrated that *W. confusa* VTT E-90392 was highly efficient in dextran synthesis in wheat sourdough, with the resulting bread exhibiting an improved shelf life, volume, and softness (Katina et al., 2009). This strain also performed well as a single starter in vegetable food fermentation for tailored texture modification (Juvonen et al., in press). Its dextransucrase gene was cloned recently and expressed in *Lactococcus lactis* (Kajala et al., 2015).

In this study, the reactions of recombinant *W. confusa* VTT E-90392 dextransucrase (WcE392-rDSR) with different acceptors were explored to complement current information on acceptor reactions and products limited to *Lc. mesenteroides* B-512F dextransucrase. WcE392-rDSR was first tested with several di- and trisaccharides to determine their relative effectiveness as acceptors and their product patterns, focusing on lactose and cellobiose, which differ only in the C4 configuration at the nonreducing end residue. Major acceptor products that formed were selected for structural analysis. The structures of the products can shed light on the interaction of different acceptor molecules with *Weissella* dextransucrases. Moreover, as *Weissella* strains and their dextransucrases have potential applications in the production of dextran and IMOs in milk-based foods (Bejar et al., 2013; Seo et al., 2007) where lactose is naturally present, it is important to identify the acceptor products formed in the presence of lactose. In this study, three products, two from a WcE392-rDSR lactose reaction mixture and one from a cellobiose reaction, were isolated and identified by NMR spectroscopy and enzymatic hydrolysis. The products were characterized by multistage mass spectrometry (MS^n), and their fragmentation pathways in positive and negative mode were determined.

2. Materials and methods

2.1. Preparation of dextransucrase and testing of acceptors

The *W. confusa* VTT E-90392 dextransucrase gene (4272 bp long, Genbank: KJ173611) was cloned into a nisin-inducible expression vector pNZ8037 for *L. lactis* NZ9800 (Kajala et al., 2015). The WcE392-rDSR was partially purified by ultrafiltration of the culture supernatant with Prep/Scale TFF (6 ft^2, cut-off 10 kDa; Millipore, Bedford, MA) and then with Amicon 8400 (cut-off 100 kDa; Millipore, Witten, Germany), followed by a 200-fold dilution with 20 mM Na-acetate buffer, pH 5.4 (Kajala et al., 2015). In SDS–PAGE analysis, one enzyme band corresponding to WcE392-rDSR was visible (data not shown). The activity of the preparation was 28.3 U/ml, as determined by the Nelson–Somogyi assay (Kajala et al., 2015), where one unit is the amount of enzyme that catalyzes the formation of 1 μmol of reducing sugar in 1 min in a 20 mM Na-acetate buffer (pH 5.4) containing 2 mM of $CaCl_2$ and 146 mM of sucrose. The protein concentration was 24.2 mg/ml, as determined with the DC Protein Assay Kit (Bio-Rad, Hercules, CA), using bovine serum albumin as the standard. Thus, the specific activity of the WcE392-rDSR preparation was 1.2 U/mg.

Various commercial di- and trisaccharides were tested as acceptors for WcE392-rDSR. Table S1 shows the concentrations of sucrose and the acceptors employed in the reactions. A WcE392-rDSR dosage of 10 U/g of sucrose was used in all the reactions. The reactions were conducted at 30 °C in 0.2 ml of a 20 mM Na-acetate buffer (pH 5.4) containing 2 mM of CaCl2 for 24 h. A sucrose:acceptor molar ratio of 4 was used, except for the lactose reaction, where two major acceptor products were observed. The effects of sucrose concentration and enzyme dosage on the formation of the two products were tested using 0.15 M lactose, a sucrose:lactose ratio of 1, 4, and 6.7, and enzyme dosages of 1, 5.5, and 10 U/g of sucrose. The condition resulting in the highest content of the two products was chosen for the lactose acceptor reaction.

2.2. High-performance anion exchange chromatography with pulsed amperometric detection (HPAEC–PAD) analysis

The WcE392-rDSR acceptor reaction mixtures and acceptor product fractions from Biogel P2 column purification (Section 2.3) were analyzed by HPAEC–PAD, equipped with a CarboPac PA-100 column (250 × 4 mm, i.d, Dionex, Sunnyvale, CA), a Decade detector (Antec Leyden, The Netherlands), a Waters 717 autosampler, and two Waters 515 pumps, as described previously (Rantanen et al., 2007). The elution (1 ml/min) was started with 75 mM of NaOH (8 min), followed by a gradient elution to 67.5 mM of NaOH and 100 mM of NaOAc (27 min).

Mono- and disaccharides in the enzymatic hydrolysates of the selected product fractions (Section 2.5) were analyzed by HPAEC–PAD, equipped with a CarboPac PA-1 column (250 × 4 mm, i.d, Dionex), a Waters 2465 pulsed amperometric detector, a Waters 2707 autosampler, and three Waters 515 HPLC pumps. The elution (1 ml/min) was started with 2 mM of NaOH (4 min), followed by the first gradient elution to 60 mM of NaOH (26 min) and the second gradient elution to 200 mM of NaOH (8 min). A solution of 300 mM of NaOH was added

postcolumn at 0.4 ml/min for PAD detection. This method was also used to quantify lactose and sucrose in reaction mixtures, as the two sugars co-eluted in the other HPAEC–PAD method described above.

Prior to the analysis, the samples were filtered with a 10 kDa of Amicon Ultra-0.5 centrifugal filter (Millipore, Billerica, MA) to remove any polymeric material. Galactose, glucose, sucrose, fructose, lactose, cellobiose (Merck, Darmstadt, Germany), leucrose (Santa Cruz Biotechnology, CA), and kojibiose (Carbosynth, Berkshire, UK) were used as standards.

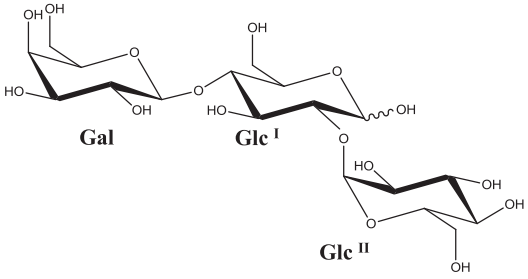
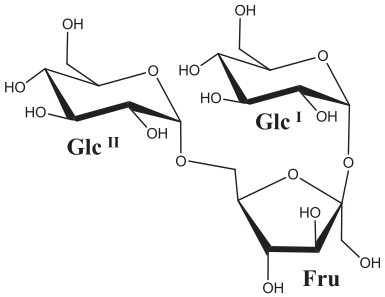
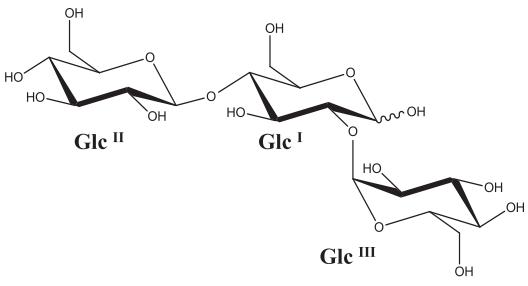
2.3. Production and isolation of the selected acceptor products

Two main products, I and II, from the WcE392-rDSR lactose reaction mixture and the main product from the cellobiose reaction, product III (Table 1), were synthesized and isolated. The acceptor reactions were conducted in 50 ml, as described in Section 2.1. After the reaction was stopped, two volumes of ethanol (Etax A, Altia, Finland) were added to the reaction mixture to remove polymeric dextran. The mixture was centrifuged (Sorvall RC5C, 28 for SLA-1500, Thermo Scientific, Waltham, MA) at 10,000 g for 20 min. The supernatant was concentrated to 2 ml using a rotatory vacuum evaporator (Heidolph Laborota 4001 Digital, Sigma–Aldrich, MO). Prior to injection into a Biogel P2 column (5 × 95 cm; Bio-Rad, Hercules, CA), the concentrate was diluted 15-fold into 5 ml, which was filtered through a 0.45 μm membrane filter (Acrodisc 13, Pall Corporation, Ann Arbor, MI). The samples were run through the column using water as the eluent at a flow rate of 0.7 ml/min. Fractions of 2 ml were collected to obtain a total volume of 400 ml. The fractions were analyzed by HPAEC–PAD (Section 2.2) and thin-layer chromatography (TLC) on a Silica Gel 60 plate (Merck, Darmstadt, Germany) with an eluent composed of ethyl acetate:acetonitrile:H_2O:1-propanol (2:7:5.5:5, v/v) (Côté & Leathers, 2005). The carbohydrates on the plate were visualized by spraying with 2% (w/v) orsinol dissolved in a solution of ethanol, H_2SO_4, and H_2O (80:10:10) and heating the plate at 100 °C for 10 min. Fractions containing the purest product of interest were pooled, and the total carbohydrate concentration of the resultant fractions was determined by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), using glucose as a standard. The fractions were used for subsequent structural analysis. They are referred to by the codes of the corresponding products of interest.

2.4. NMR characterization of the selected products

For the structural elucidation of the products of interest, 1D ^1H and 2D (multiplicity-edited HSQC, DQFCOSY, TOCSY, and HMBC) nuclear magnetic resonance (NMR) spectroscopy experiments were carried out. The samples were freeze-dried and redissolved in D_2O (MagniSolv, Merck KGaA, Darmstadt, Germany) to a carbohydrate concentration of 4–8 mg/ml, and they were analyzed in NMR tubes (5 mm, Wilmad Ultra-Imperial, Sigma–Aldrich) on a 600 MHz Bruker Avance III NMR spectrometer (Bruker BioSpin, Rheinstetten, Germany) equipped with a QCI cryoprobe. The measurements were performed at 22 °C. However, one anomeric

Table 1
Nomenclature and structure of products I and II from the WcE392-rDSR lactose reaction mixture and those of product III from the WcE392-rDSR cellobiose acceptor reaction. Sugar residue annotations are used in the interpretation of the NMR data.

Substrates (mM)		Product code	Product nomenclature	Product structure
Sucrose 1000	Lactose 150	I	2^{Glc}-α-Glc*p*-lactose	
		II	Isomelezitose/6^{Fru}-α-Glc*p*-sucrose	
Sucrose 200	Cellobiose 50	III	2^1-α-Glc*p*-cellobiose	

proton signal of products I and III (Table 1) overlapped with the residual water signal at 22 °C. Thus, the 1D ^{1}H and HSQC spectra were re-run at 16 °C. In the 1D ^{1}H experiments, the residual water signal was suppressed by 4 s volume selective presaturation (so called 1D NOESY presaturation) using Bruker's pulse program noesygppr1d. In the TOCSY experiments, a DIPSI2 sequence and a mixing time of 80 ms were used. The HMBC experiments were optimized for 8 Hz (62.5 ms) long-range coupling constants. In the two-dimensional experiments, matrices of 2 K × 256 (2 K × 512 in HMBC) data points were collected and zero filled once in F1. A π/2-shifted sine bell weighting function was applied in both dimensions prior to the Fourier transformation. The chemical shifts of ^{1}H (δ_H) and ^{13}C (δ_C) were referenced to acetone (δ_H 2.225 ppm and δ_C 31.50 ppm, respectively).

2.5. Enzymatic hydrolysis of the isolated products

The three isolated products were hydrolyzed with specific enzymes. All the hydrolysis reactions were conducted overnight at 30 °C in 20 mM of Na-acetate buffer (pH 5). The enzymes were inactivated by boiling for 10 min after the treatments. Products I, II, and III were all treated with 25 U/mg of carbohydrate of *Aspergillus niger* α-glucosidase (Megazyme, Wicklow, Ireland). Products I and III were also treated with 50 U/mg of carbohydrate of *A. niger* β-galactosidase (Megazyme) and 75 U/mg of carbohydrate of *Agrobacterium* sp. β-glucosidase (Megazyme), respectively. Fractionated product II was treated with 200 U/mg of carbohydrate of *Chaetomium erraticum* dextranase (Sigma–Aldrich). The susceptibilities of product II and raffinose (Sigma–Aldrich) to yeast invertase (β-fructosidase, Megazyme) were compared under 100 U/mg of carbohydrate. The mono- and disaccharides in the hydrolysates were analyzed by HPAEC–PAD (Section 2.2).

2.6. MS^n analysis of the selected products

The unlabeled products I, II, and III were analyzed by electrospray ionization multistage ion trap mass spectrometry (ESI-IT-MS^n). The fragmentation patterns of the trisaccharides were studied in both negative and positive mode ($[M+Cl]^-$ and $[M+Li]^+$, respectively) using an Agilent XCT Plus Ion Trap mass spectrometer with an electrospray ion source (Agilent Technology, Palo Alto, CA). A 1–2 µl of sample was diluted in 400 µl of methanol–water-formic acid (50:49:1, v:v:v), and 0.5 µl of 10 mg/ml ammonium chloride or lithium acetate were added for adduct ion formation ($[M+Li]^+$ and $[M+Cl]^-$, respectively). The samples were introduced to the ion source with a direct infusion syringe pump at a flow rate of 5 µl/min. The ionization parameters were as previously reported (Maina et al., 2013), except ±3200 V was used for capillary voltage. The ion trap parameters were automatically adjusted by the mass of the analyte.

The WcE392-rDSR lactose reaction mixture was also produced using $^{13}C_6^{glc}$sucrose to enable complete characterization of the fragmentation pattern of 2^{Glc}-α-Glc*p*-lactose and isomelezitose by ESI-IT-MS^n. The $^{13}C_6^{glc}$-labeled products were prepared from [UL-$^{13}C_6^{glc}$] sucrose (Omicron Biochemicals Inc. IN) and lactose in 40 µl, as described in Section 2.1, except that a lower WcE392-rDSR dosage was used with 5.5 U/g of sucrose. The dextran was removed by filtering with a 10 kDa Amicon Ultra-0.5 centrifugal filter (Millipore). The filtrate was diluted to 1 ml volume, and monosaccharides and buffer salts were removed using a Hypersep Hypercarb Porous Graphitized Carbon column (Thermo Scientific) (Packer, Lawson, Jardine, & Redmond, 1998). Oligosaccharides were eluted with a solution containing 50% (v/v) of acetonitrile and 0.1% (v/v) trifluoroacetic acid. The samples were dried under nitrogen gas for 30 min and freeze-dried overnight. The purified reaction mixture was diluted to 40 µl in distilled water and analyzed by the ESI-MS^n method, as described in the previous paragraph.

3. Results

3.1. Acceptor reactions of WcE392-rDSR

The action of WcE392-rDSR on several glucose-containing di- and trisaccharides was evaluated. Maltose was not included in the series, as it is known to be a good acceptor for this dextransucrase (Shukla et al., 2014). In all the reactions, a large amount of fructose was released, and dextran was produced. Judging by the product peak area in the HPAEC–PAD chromatograms, the best acceptors of those tested were isomaltose (α-D-Glc*p*-(1→6)-D-Glc*p*) (Fig. S1a), maltotriose (α-D-Glc*p*-(1→4)-α-D-Glc*p*-(1→4)-D-Glc*p*) (Fig. S1b), and nigerose (α-D-Glc*p*-(1→3)-D-Glc*p*) (Fig. S1c). A range of products was detected in these reaction mixtures, indicating that the acceptor products could be further elongated. Lactose (β-D-Gal*p*-(1→4)-D-Glc*p*) (Fig. 1a) and cellobiose (β-D-Glc*p*-(1→4)-D-Glc*p*) (Fig. 1b) were relatively moderate acceptors, and melibiose (α-D-Gal*p*-(1→6)-D-Glc*p*), laminaribiose (β-D-Glc*p*-(1→3)-D-Glc*p*), and forkose (α-D-Glc*p*-(1→4)-[α-D-Glc*p*-(1→6)]-D-Glc*p*) (Hansen et al., 2008), were poor acceptors for WcE392-rDSR (data not shown).

In the lactose reactions with a fixed lactose concentration, oligosaccharide production increased with an increase in the sucrose concentration (Fig. S2). The dextransucrase concentration positively influenced the production of oligosaccharides but to a lesser extent than the concentration of sucrose. The highest concentration of the two main products (I and II in Fig. 1a) was obtained with the highest sucrose and dextransucrase concentrations tested (Fig. S2b). Under this condition, all initial sucrose and 27% of initial lactose were consumed after 24 h of reaction. Assuming that lactose was mostly converted to 2^{Glc}-α-Glc*p*-lactose, its yield can be estimated to be around 20 mg/ml.

3.2. Production and isolation of the selected acceptor products

Products I and II from the lactose reaction mixture and product III from the cellobiose reaction mixture were isolated by gel filtration chromatography. The fractions selected contained approximately 2–4 mg of carbohydrate, as determined by a phenol–sulfuric acid assay. The HPAEC–PAD analysis showed that each fraction was composed mainly of the product of interest. TLC analysis (data not shown) confirmed that there was one major compound present in the selected fractions. All three products ($[M+Cl]^-$ ions) exhibited an *m*/*z* value of 539 (Fig. 1c), demonstrating that they were trisaccharides.

3.3. NMR characterization of the selected products

Each product fraction was analyzed by NMR using 1D ^{1}H, multiplicity-edited HSQC, DQFCOSY, TOCSY, and HMBC experiments. The anomeric proton regions in 1D ^{1}H spectra of products I and III were similar, and they were distinctly different from that of product II (Fig. 2). The edited HSQC, TOCSY, and HMBC spectra of the three products are shown in Figs. S3–S5.

3.3.1. Structure of products I and III

In the 1D ^{1}H spectra of products I and III, six doublets in the anomeric proton region were observed (Fig. 2), indicating resonances for both the α and β anomers, respectively. Taking product III, for example, the signals at δ_H 5.44 ppm (d J_{1-2} 3.5 Hz) and δ_H 4.81 ppm (d J_{1-2} 8.0 Hz) corresponded to α and β anomeric protons

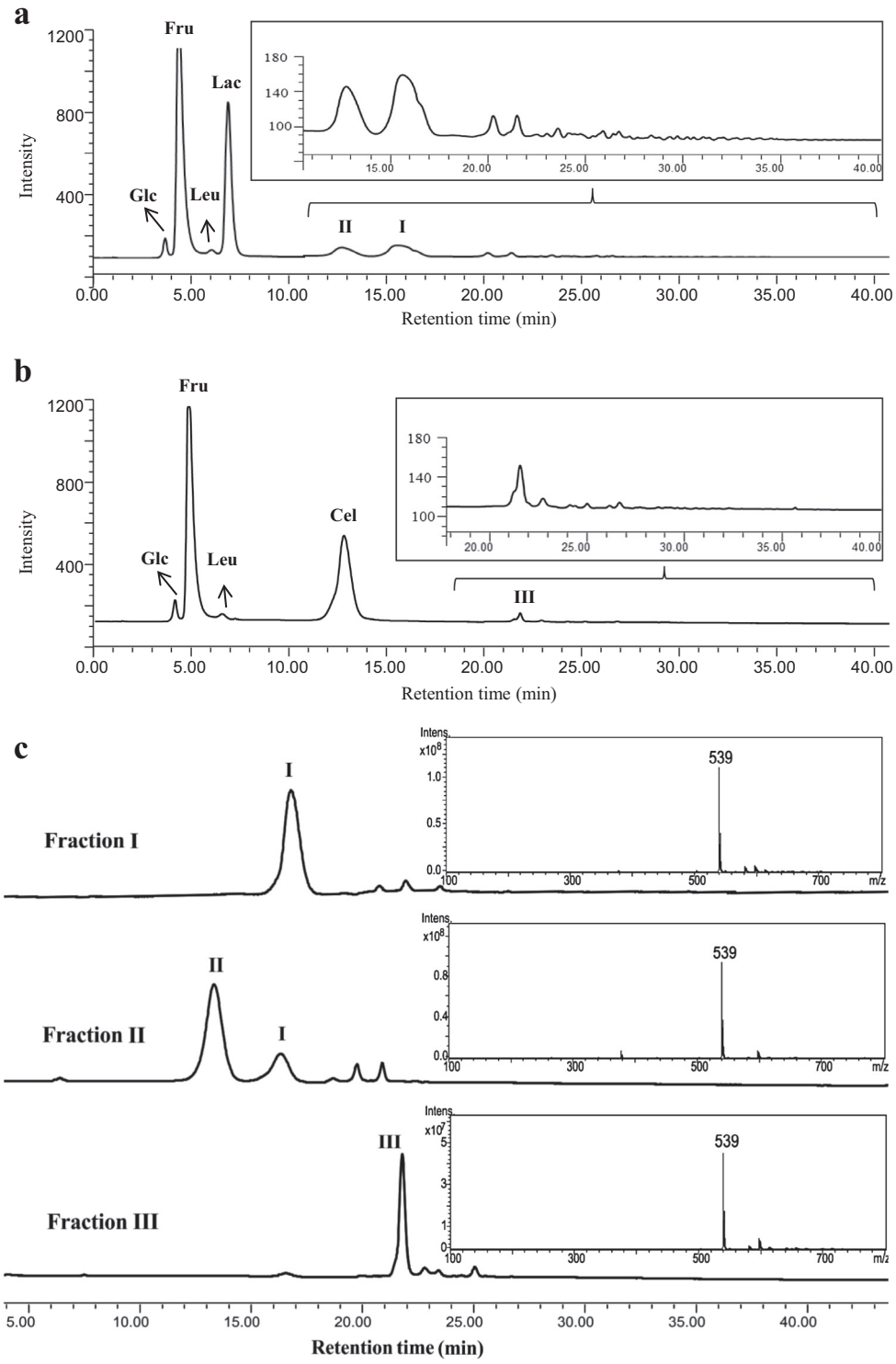


Fig. 1. HPAEC–PAD profiles of the (a and b) 24 h reaction mixtures of WcE392-rDSR (10 U/g sucrose) with (a) 150 mM lactose and 1 M sucrose and (b) 50 mM cellobiose and 200 mM sucrose and those of the (c) Biogel P2 fractions of isolated products I and II from the lactose reaction mixture and product III from the cellobiose reaction mixture. Each fraction is referred to by the product code of its major component. The labeled peaks are: Glc, glucose; Fru, fructose; Leu, leucrose; Lac, lactose; and Cel, cellobiose. The inset in (a) and (b) shows the zoomed in chromatogram of the acceptor product area. The insets in (c) show the ESI-MS spectrum ($[M+Cl]^-$) of each product fraction.

of the reducing glucopyranosyl residue, respectively. The chemical shifts of the two other residues in the trisaccharide were affected by mutarotation equilibrium, resulting in two sets of resonances for each residue. The signals at δ_H 4.52 ppm (d J_{1-2} 7.8 Hz) and δ_H 4.51 ppm (d J_{1-2} 7.9 Hz) correspond to the anomeric proton of the β-(1→4)-linked glucopyranosyl residue of the cellobiose acceptor molecule. The last two anomeric signals at δ_H 5.10 and δ_H 5.37 ppm (both d J_{1-2} 3.8 Hz) are from an α-(1→2)-linked glucopyranosyl residue (Fig. 2). Evidence for α-(1→2)-linkage was observed in the HMBC correlation peaks: between Glc^{I}-C2 (δ_C 76.84 ppm) and Glc^{III}-H1 (δ_H 5.10 ppm), and Glc^{III}-C1 (δ_C 97.70 ppm) and Glc^{I}-H2 (δ_H 3.67 ppm) for the α anomer; and

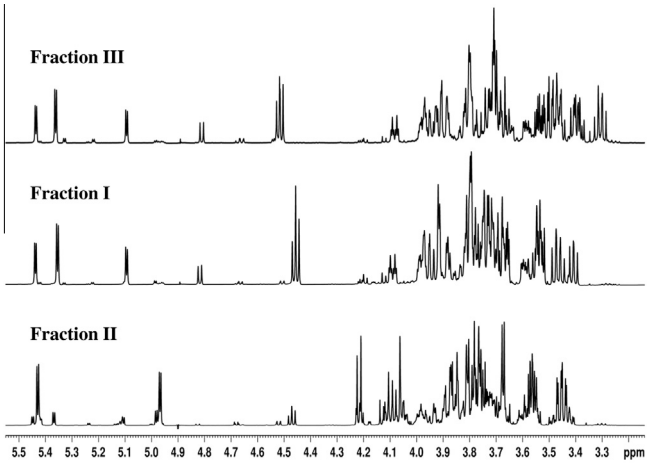


Fig. 2. 1D ^{1}H NMR spectra of the product fraction III from the WcE392-rDSR cellobiose reaction mixture and the product fractions I and II from the lactose reaction mixture. The spectra were measured at 600 MHz in D_2O at 16 °C.

between Glc^{I}-C2 (δ_C 79.60 ppm) and Glc^{III}-H1(δ_H 5.37 ppm), and Glc^{III}-C1 (δ_C 99.20 ppm) and Glc^{I}-H2 (δ_H 3.41 ppm) for the β anomer (Fig. S3). Thus, product III was identified as α-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→4)]-D-glucopyranose (2^{1}-α-Glc*p*-cellobiose). The full NMR assignments are shown in Table S2.

Similarly, product I was identified as α-D-glucopyranosyl-(1→2)-[β-D-galactopyranosyl-(1→4)]-D-glucopyranose, (2^{Glc}-α-Glc*p*-lactose). The 1D ^{1}H and 2D NMR spectra are shown in Figs. 2 and S4, and the full ^{1}H and ^{13}C NMR assignments are shown in Table S2. Thus, both products I and III contain a glucosyl residue that is α-(1→2)-linked to the reducing end of the acceptor disaccharide (lactose and cellobiose, respectively). The ratio of α and β anomers (39% and 61%, respectively) for both trisaccharides was determined from the integrals of the anomeric protons of the α-(1→2)-linked glucopyranosyl residue, as the β anomeric signal of the reducing end residue was partially suppressed with the residual water signal.

3.3.2. Structure of product II

The product fraction II contained mostly product II and a small amount of impurities, mainly product I (Fig. 1c). The NMR signals from the impurities were minor and did not interfere with the chemical shift assignment of product II. There were two anomeric protons at δ_H 5.42 ppm (d J_{1-2} 3.9 Hz) and δ_H 4.96 ppm (d J_{1-2} 3.7 Hz), both with an α configuration (Fig. 2 and Table S3). The presence of only two anomeric protons for a trisaccharide indicated that one of its sugar units has a quaternary anomeric carbon and no corresponding anomeric proton. As sucrose was the only sugar in the reaction system with this feature, product II was inferred to contain sucrose, which was supported by comparing its ^{13}C assignments with that of sucrose (Table S3). The third residue exhibited resonances typical of a terminal α-glucopyranosyl unit. The NMR data showed that it was α-(1→6)-linked to the fructofuranosyl residue of sucrose, as manifested by the downfield shift of fructofuranosyl C6 signals in the edited HSQC (Fig. S5a). The linkage was also confirmed by correlation peaks in the HMBC spectrum between Fru-C2 (δ_C 104.71 ppm) and Glc^{I}-H1 (δ_H 5.42 ppm), Fru-C6 (δ_C 70.08 ppm) and Glc^{II}-H1 (δ_H 4.96 ppm), and Glc^{II}-C1 (δ_C 99.58 ppm) and Fru-H6 (δ_H 4.06 and 3.76 ppm) (Fig. S5c).

Product II was consequently identified as an α-D-glucopyranosyl-(1→2)-[α-D-glucopyranosyl-(1→6)]-β-D-fructofuranoside (6^{Fru}-α-Glc*p*-sucrose/2^{Fru}-α-Glc*p*-isomaltulose), which is called isomelezitose. It has no reducing end and thus no α/β anomers. The full assignments are shown in Table S3.

3.4. Structure confirmation by enzymatic hydrolysis

The product fractions I, II, and III were selectively digested with specific enzymes and then analyzed by HPAEC–PAD (Fig. 3). The compounds that formed were identified based on their retention time against standards.

The α-(1→2)-linkage in products I and III (Fig. 3a and c) was hydrolyzed by α-glucosidase, generating glucose and the corresponding acceptor (lactose and cellobiose, respectively). The β-(1→4)-linkage in product I was hydrolyzed by β-galactosidase (Fig. 3d). As a result, galactose and kojibiose (α-D-Glc*p*-(1→2)-D-Glc*p*) were obtained, as expected. The observed glucose release was due to the side reaction of the β-galactosidase preparation on the α-(1→2)-linked glucosyl residue. The formation of glucose by the β-galactosidase preparation was also observed for product III and pure kojibiose (data not shown). Although product III was not very susceptible to hydrolysis by the β-glucosidase used (Fig. 3e), a small amount of the product was converted to glucose and kojibiose. The above results further support the notion that products I and III contain a glucosyl unit, which is α-(1→2)-linked to the reducing end of their acceptors.

Product II was hydrolyzed to glucose and sucrose by α-glucosidase, and the sucrose appeared to be then converted to glucose and fructose (Fig. 3b). This confirms that the product contains sucrose. However, it was resistant to dextranase (data not shown), which requires two consecutive α-(1→6)-linked glucosyl units for its function (Maina et al., 2011). Product II was also not hydrolyzed by invertase, whereas raffinose (α-D-Gal*p*-(1→6)-α-D-Glc*p*-(1→2)-β-D-Fru*f*) was hydrolyzed to fructose and melibiose (α-D-Gal*p*-(1→6)-D-Glc*p*) (data not shown), indicating that fructose is further substituted in product II. This concurs with the structure of product II having a glucosyl unit, which is α-(1→6)-linked to the fructosyl residue, whereas the galactosyl unit in raffinose is α-(1→6)-linked to the glucosyl residue of sucrose.

3.5. MS^{n} analysis of the selected products

The three identified trisaccharide acceptor products were analyzed by ESI-IT-MS^{n} using negative and positive ionization. Their MS^{n} spectra are shown in Figs. S6–S8. The spectra were difficult to fully interpret because the samples seemed to contain impurities that were isomeric with the products. Therefore, some fragment ions did not fit the structural features identified with the NMR data. Nonetheless, the fragmentation pattern of product I from lactose and III from cellobiose acceptor reactions were similar, indicating that they had comparable structural features. To characterize the fragment ions, the lactose acceptor reaction was prepared using $^{13}C_6^{glc}$-labeled sucrose. As the labeled products I and II had different molecular masses, they could be analyzed directly from the reaction mixture without fractionation. The $^{13}C_6^{glc}$ labeling would also differentiate the isomeric glycosidic cleavage fragment ions and allow these ions to be analyzed separately at the MS^3 stage.

The HPAEC–PAD profile of the $^{13}C_6^{glc}$-labeled product mixture shows the formation of oligosaccharides in the presence of [UL-$^{13}C_6^{glc}$] sucrose and lactose (Fig. S9). In the negative MS spectrum (Fig. S9), the trisaccharides (DP3) I and II were identified as *m*/*z* 545 and *m*/*z* 551, respectively, with the former having one ^{13}C-labeled glucose unit and the latter having two ^{13}C-labeled glucose units. The reaction mixture also contained tetrasaccharides (DP4), pentasaccharides (DP5), and hexasaccharides (DP6). Interestingly, all the products contained at least one unlabeled unit. Also notably, products with two unlabeled hexose units were

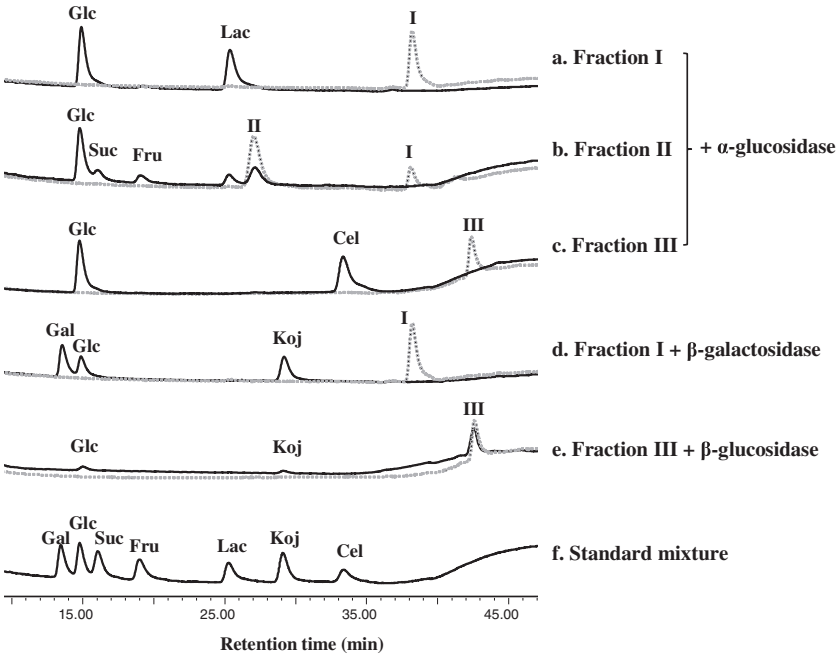


Fig. 3. HPAEC–PAD profiles of the (a–e) product fractions before (dashed lines) and after (solid lines) specific enzymatic digestion and that of the (f) standard mixture. Gal: galactose, Glc: glucose, Suc: sucrose, Fru: fructose, Lac: lactose, Koj: kojibiose, and Cel: cellobiose.

identified only in the DP3 and DP4 (*m*/*z* 545 and *m*/*z* 713) products. The same products were observed in positive-mode MS (data not shown). The $^{13}C_6^{glc}$-labeled trisaccharides I and II were further analyzed with ESI-MS^n to characterize their fragmentation patterns.

3.5.1. MS^n analysis of $^{13}C_6^{glc}$-labeled product I

The MS^n spectra of $^{13}C_6^{glc}$-labeled product I are shown in Fig. 4. The major fragment ion in the negative ion MS^2 spectrum (Fig. 4a) of product I (*m*/*z* 545) was the $^{0,4}X_0$-H_2O ion (*m*/*z* 431), indicating the presence of a reducing end (Zhu & Cole, 2001). Further MS^n spectra analysis of Y-ions in the negative mode was not possible due to their low abundance.

Contrary to the negative ion MS^2 spectrum, the $^{0,4}X_0$ cross-ring fragment ion was not observed in the positive ion MS^2 spectrum (Fig. 4b). Nonetheless, in the positive ion MS^3 spectrum of the $Y_{1\alpha}$ ion (*m*/*z* 349) originating from the loss of a ^{13}C-labeled hexopyranosyl residue (Fig. 4c), cross-ring fragments ($^{0,2}A_2$ and $^{2,4}A_2$, loss of 60 and 120 Da, respectively) diagnostic of a (1→4)-linkage were produced. Furthermore, the Y_1 ion (*m*/*z* 355) originating from the loss of an unlabeled hexopyranosyl residue (Fig. 4d) produced a cross-ring fragment ion, $^{0,2}X_0$ (*m*/*z* 235, loss of 120 Da), that was diagnostic of a (1→2)-linkage (Hernández-Hernández, Calvillo, Lebrón-Aguilar, Moreno, & Sanz, 2012). These findings support product I being nonlinear 2^{Glc}-α-Glc*p*-lactose.

3.5.2. MS^n analysis of $^{13}C_6^{glc}$-labeled product II

In negative ionization MS^2 (Fig. 5a), the $^{13}C_6^{glc}$-labeled product II (*m*/*z* 551) fragmented only by the loss of hydrogen chloride (HCl) produced a deprotonated molecular ion, $[M-H]^-$ (*m*/*z* 515). It did not produce either a glycosidic bond or cross-ring cleavage fragments, suggesting the absence of a reducing end. The $[M-H]^-$ ion was isolated and fragmented at the MS^3 stage. The MS^3 spectrum (Fig. 5b) showed an interesting cross-ring fragment ion at *m*/*z* 239. A previous study observed a similar fragment ion from the negative MS^2 spectrum of 6-kestose (β-D-Fru*f*-(2→6)-β-D-Fru*f*-(2→1)-α-D-Glc*p*) (Harrison, Xue, Lane, Villas-Boas, & Rasmussen, 2012). This fragment ion (named here as $^{0,2}A$-H_2O ion) was formed by cross-ring cleavage of the middle fructofuranosyl unit.

The ion at *m*/*z* 395 (Fig. 5b) was a cross-ring fragment ion formed by neutral loss of 120 Da from an unlabeled hexosyl unit. The spectrum also showed a minor peak at *m*/*z* 353, which was a disaccharide fragment ion with two ^{13}C-labeled glucose units, further indicating the loss of an unlabeled hexosyl unit. The cross-ring fragment ion produced from this disaccharide was also seen at *m*/*z* 273 (loss of Hex + 62 Da + H_2O). To our knowledge, it is not possible that these three ions could be formed from the $^{13}C_6^{glc}$-labeled product II, indicating a small portion of impurity that consisted of two consecutive labeled glucosyl units and one unlabeled hexosyl unit at the reducing end.

Z- and Y-ions were the most abundant ions in the positive ion MS^2 spectrum of product II (Fig. 5c). As a nonreducing trisaccharide, product II did not produce cross-ring cleavage ions from terminal units. The positive-mode MS^2 spectrum showed a small cross-ring fragment peak at *m*/*z* 433, indicating the loss of 90 Da from an unlabeled hexosyl unit. The corresponding ion was not observed in the *m*/*z* 511 MS^2 spectra of unlabeled product II (Fig. S7c) or in α-glucosidase-treated unlabeled product II (Fig. 3b, MS data not shown), suggesting the presence of impurity as observed in negative MS^3 spectrum. The Y-ions were further fragmented in MS^3 (Fig. 5d). Fragmentation of fructose in $Y_{1\alpha}$ produced $^{0,1}A_2$, $^{0,2}A_2$, and $^{0,3}A_2$ cross-ring ions. The fragmentation pattern was similar to that of isomaltulose in positive ionization (Lee, Valentine, Reilly, & Clemmer, 2012) and therefore signified a (1→6)-linked fructose unit at the reducing end. Although both Y_1 and $Y_{1\alpha}$ were isomeric, the observed fragments were only from $Y_{1\alpha}$, as Y_1 was a nonreducing disaccharide (sucrose) and therefore did not produce fragments. The interpretation of the MS^n spectra thus confirmed the proposed structure of product II as 6^{Fru}-α-Glc*p*-sucrose.

4. Discussion

The selectivity and product patterns of WcE392-rDSR on different acceptors were similar to those reported for *Lc. mesenteroides*

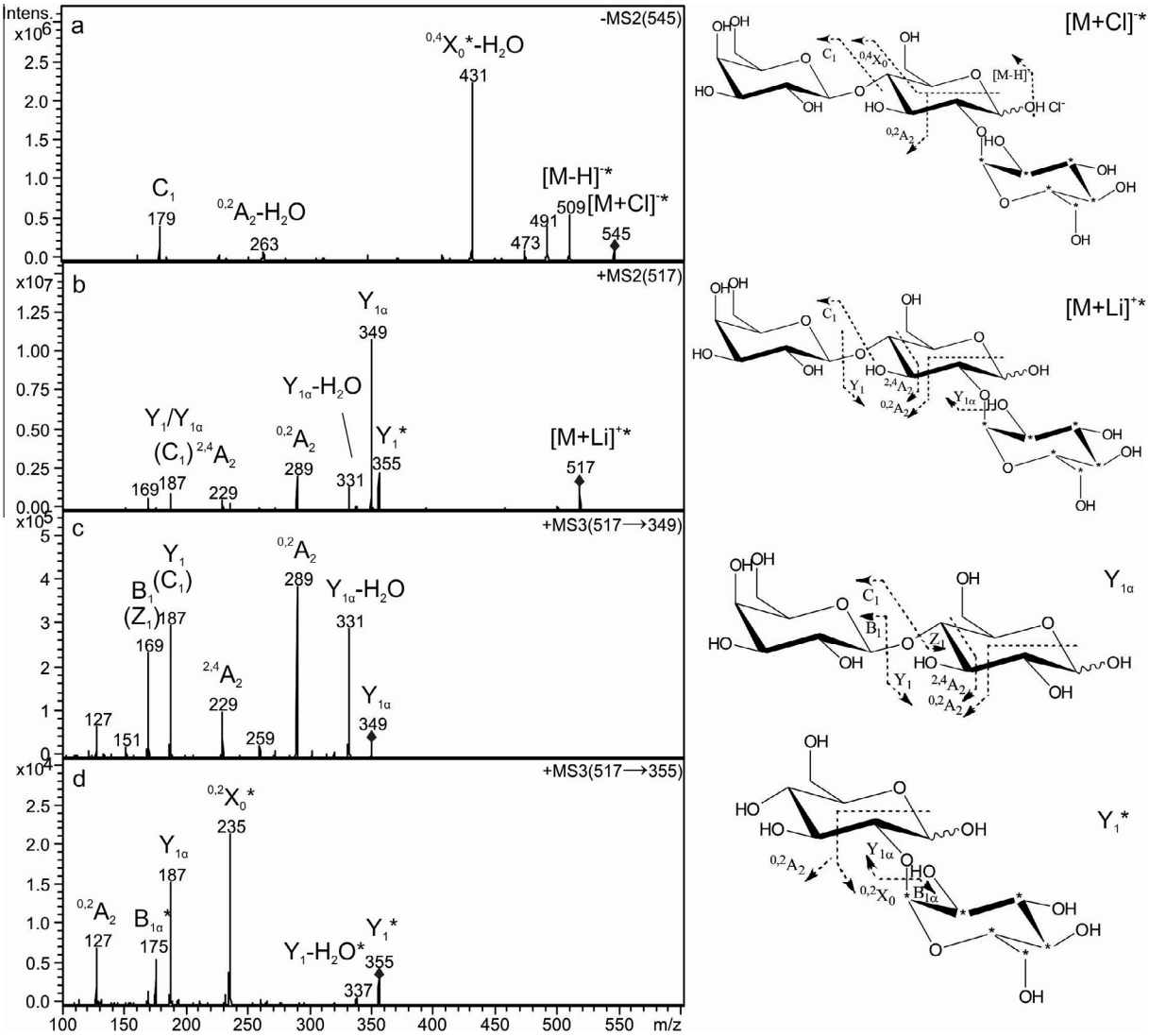


Fig. 4. MS^n spectra of $^{13}C_6^{glc}$-labeled acceptor product I. (a) Negative ion MS^2 of $[M+Cl]^-$ *m/z* 545, (b) positive ion MS^2 of $[M+Li]^+$ *m/z* 517, (c) positive ion MS^3 of $Y_{1\alpha}$ *m/z* 517 → 349, and (d) positive ion MS^3 of Y_1 *m/z* 517 → 355. All the fragment ions in positive ionization are lithium adduct ions. In the spectra, *: the number of $^{13}C_6^{glc}$-labeled units in the compound. In the structural model, *: the ^{13}C carbon. The fragment ions are named according to Domon and Costello (1988).

B-512F dextransucrase based on the tests with isomaltose, maltotriose, nigerose, lactose, and cellobiose (Robyt, 1995; Robyt & Eklund, 1983). The unusual acceptors tested in this study (i.e., melibiose, laminaribiose, and forkose) were not effective acceptors for WcE392-rDSR. The low activity toward forkose (α-D-Glc*p*-(1→4)-[α-D-Glc*p*-(1→6)]-D-Glc*p*) was in line with an earlier report on maltooligosaccharides carrying an α-(1→6)-linked glucosyl unit on the reducing end residue (Fu & Robyt, 1990). The dextransucrase from the *W. confusa* Cab3 strain produced similar IMOs from maltose as did the dextransucrase from *Lc. mesenteroides* B-512F (Shukla et al., 2014). Dextrans produced by *W. confusa* are also reported to be structurally similar to dextran from *Lc. mesenteroides* B-512F (Maina et al., 2011; Shukla et al., 2014). The specificity of *W. confusa* dextransucrases seems thus similar to that of the widely studied *Lc. mesenteroides* B-512F dextransucrase, although the sequence of WcE392-rDSR (Genbank: KJ173611) (Kajala et al., 2015) bears only 52% amino acid similarity to *Lc. mesenteroides* NRRL B-512F dextransucrase (Genbank: AAD10952) on a 98% query coverage based on BLAST analysis.

For both *Lc. mesenteroides* B-512F dextransucrase and WcE392-rDSR, the main acceptor product of β-(1→4)-linked lactose or cellobiose was a trisaccharide with a glucosyl residue α-(1→2)-linked to the acceptor's reducing end, unlike α-linked disaccharide acceptors, where the glucosyl residue is usually α-(1→6)-linked to the nonreducing end (Robyt, 1995). This indicates that the linkage specificity of dextransucrase is dependent on the acceptor's structure, which dictates its binding mode at the active site. Ruiz-Matute et al. (2011) showed that *Lc. mesenteroides* B-512F dextransucrase also produced a minor α-(1→6)-linked linear trisaccharide from cellobiose, besides the major α-(1→2)-branched product. The former was further elongated via α-(1→6)-linkages to DP6, whereas the latter was not. The small peaks of higher degrees of polymerization observed for the WcE392-rDSR cellobiose reaction mixture could thus be from a linear series. However, neither the present study nor that of Díez-Municio et al. (2012a) identified a similar series of oligosaccharides that are lactose-based.

Previous *in vitro* studies demonstrated that kojibiose and lactose- and cellobiose-based α-(1→2)-branched trisaccharides were selective toward probiotic bacteria due to their α-(1→2)-linkage, and they were proposed as potential prebiotics (García-Cayuela et al., 2014; Ruiz-Matute et al., 2011; Sanz,

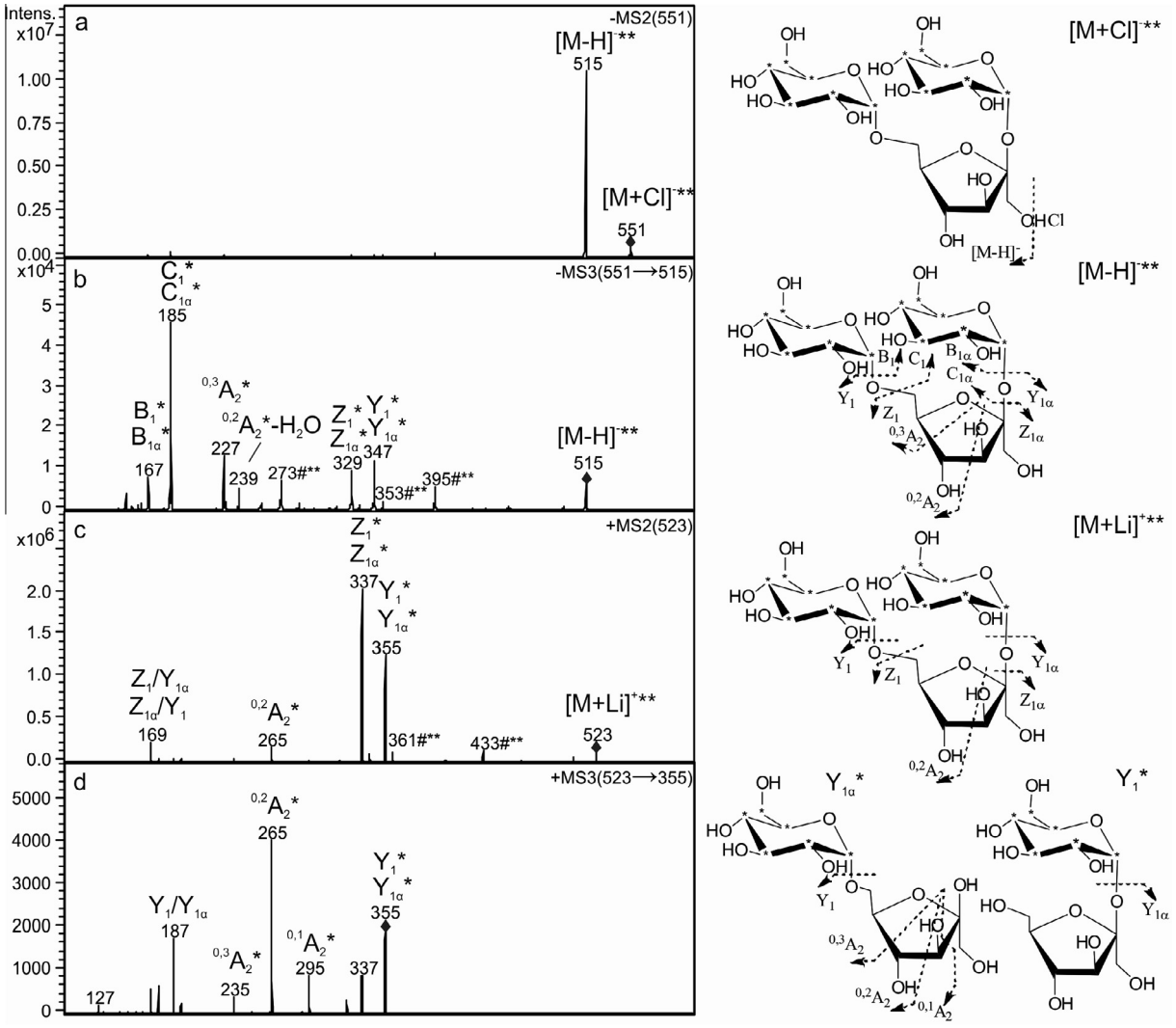


Fig. 5. MS^n spectra of $^{13}C_6^{glc}$-labeled acceptor product II. (a) Negative ion MS^2 of $[M+Cl]^-$ *m/z* 551, (b) negative ion MS^3 of $[M-H]^-$ *m/z* 551 → 515, (c) positive ion MS^2 $[M+Li]^+$ *m/z* 523, (d) positive ion MS^3 of $Y_{1\alpha}$ and Y_1 *m/z* 523 → 355. All the fragment ions in positive ionization are lithium adduct ions. In the spectra, *: the number of the $^{13}C_6^{glc}$-labeled units in the compound, #: the fragment ions of unknown trisaccharides. In the structural model, *: the ^{13}C carbon. The fragment ions named according to Domon and Costello (1988).

Gibson, & Rastall, 2005). Recently, the dextransucrase acceptor product 2^{Glc}-α-Glc*p*-lactose was used as a precursor for the synthesis of kojibiose, whose applications as a bioactive compound are limited by its low availability (Díez-Municio, Montilla, Moreno, & Herrero, 2014). Dextransucrase acceptor reactions may thus provide an alternative to synthesize potential prebiotics using readily available and inexpensive substrates, such as sucrose and lactose. They can also be exploited for *in situ* production of oligosaccharides in fermented milk products.

In the synthesis of 2^{Glc}-α-Glc*p*-lactose with *Lc. mesenteroides* B-512F dextransucrase (0.8 U/ml), the sucrose and lactose concentrations of 0.88 M (ratio of 1) were selected, in order to decrease the formation of dextran and leucrose (Díez-Municio et al., 2012a). The maximum yield of 2^{Glc}-α-Glc*p*-lactose was achieved after 24 h reaction, after which the yield remained constant. This was consistent with the gradual decrease of lactose until 36% of the initial content was consumed after 24 h. The production of 2^{Glc}-α-Glc*p*-lactose in moles was slightly lower than the loss of lactose (Díez-Municio et al., 2012a). In the present study, however, only 27% of the initial lactose was consumed at the end of the reaction. The lower lactose conversion efficiency could be explained by the lower initial lactose concentration used (0.15 M). In fact, Díez-Municio et al. attributed their high yield of 2^{Glc}-α-Glc*p*-lactose to the high initial concentrations of lactose and sucrose (2012a). With the fixed lactose concentration used in the present study, the production of 2^{Glc}-α-Glc*p*-lactose increased with increasing sucrose and dextransucrase concentrations. The production of isomelezitose, which was independent of lactose, was also enhanced by higher concentrations of sucrose and dextransucrase. To the best of our knowledge, this is the first report of isomelezitose from a dextransucrase reaction. Díez-Municio et al. (2012a) described a small amount of an unknown trisaccharide product, which may have been isomelezitose.

Isomelezitose is a potential nutraceutical (Görl, Timm, & Seibel, 2012). In a previous study, it was obtained from the reaction of alternansucrase with fructose and accounted for 5% of the total trisaccharides isolated (Côté, Sheng, & Dunlap, 2008). The other two trisaccharides, glucosylated on the nonreducing end of leucrose (α-D-Glc*p*-(1→5)-D-Fru*p*) and isomaltulose (α-D-Glc*p*-(1→6)-D-Fru*f*), accounted for 90% and 5%, respectively (Côté et al., 2008). Two possible pathways have been proposed for the synthesis of isomelezitose. One involves glucosyl transfer to the 2-OH of

the reducing end fructose residue of isomaltulose, resulting in the formation of a sucrose-type (β-D-Fru*f*-(2→1)-α-D-Glc*p*)-linkage. Isomaltulose is present as an acceptor product of fructose. The other pathway involves the transfer of a glucosyl residue to the 6-OH of the fructofuranosyl residue of another sucrose molecule. The evidence available seems to favor the isomaltulose-derived pathway. First, the formation of a sucrose-type linkage on lactulose (β-D-Gal*p*-(1→4)-D-Fru*f*) and maltulose (α-D-Glc*p*-(1→4)-D-Fru*f*) catalyzed by *Lc. mesenteroides* dextransucrase (Demuth, Jördening, & Buchholz, 2002; Díez-Municio et al., 2012b) illustrates that a similar sucrose-type linkage could be formed on isomaltulose to produce isomelezitose. Second, when isomaltulose was used as an acceptor, *Lc. mesenteroides* dextransucrase produced a series of oligosaccharides that were elongated via α-(1→6)-linkages. However, a major imbalance between the consumption of isomaltulose and the yield of oligosaccharides indicates that other compounds are also produced from isomaltulose (Barea-Alvarez, Benito, Olano, Jimeno, & Moreno, 2014). On the other hand, sucrose is considered an initiator of dextran synthesis, and sucrose-based oligosaccharides have been described (Moulis et al., 2006). However, added glucosyl residues are elongated on the glucosyl moiety of sucrose, not on the fructosyl residue, as is the case with isomelezitose. Based on the above evidence, we hypothesize that isomelezitose is an acceptor product of isomaltulose, that is formed when fructose released from sucrose acts first as an acceptor. Interestingly, the sucrose analogs β-D-Gal*p*-(1→4)-β-D-Fru*f*-(2→1)-α-D-Glc*p* (lactulosucrose) and α-D-Glc*p*-(1→4)-β-D-Fru*f*-(2→1)-α-D-Glc*p* derived from lactulose and maltulose, respectively, are formed first and consumed later (Demuth et al., 2002; Díez-Municio et al., 2012b). Thus, they are considered donor substrates for dextransucrase (Demuth et al., 2002; Díez-Municio et al., 2012b). It is not known whether dextransucrase can also utilize isomelezitose as a donor substrate. Further studies on the kinetics of the synthesis of oligosaccharides in dextransucrase-catalyzed reactions may help to understand the mechanism underlying the synthesis of isomelezitose.

The NMR spectral data obtained for 2^{Glc}-α-Glc*p*-lactose, 2^{1}-α-Glc*p*-cellobiose, and isomelezitose were consistent with earlier reports on these trisaccharides (Argüello Morales et al., 2001; Côté et al., 2008; Díez-Municio et al., 2012a). The NMR spectra of 2^{1}-α-Glc*p*-cellobiose were fully assigned for the first time in this study. The trisaccharides were also analyzed by ESI-IT-MS^n to examine whether they would produce structure-diagnostic fragment ions in MS^n spectra, so that only the MS method would be needed for identification in future studies. To our knowledge, no MS^n study of oligosaccharides containing a 2,4-*O*-disubstituted residue has been reported earlier. ^{13}C isotope labeling enabled us to fragment separately the disaccharide product ions (Y-ions) from both ends of the branched 2^{Glc}-α-Glc*p*-lactose. As expected, the fragmentation patterns of the Y-ions confirmed the presence of (1→4)- and (1→2)-linkages. The combination of both positive and negative ionization techniques was demonstrated to be beneficial in the analysis of carbohydrates containing a 2,4-*O*-disubstituted reducing end residue. The negative ion MS^2 spectra of the branched 2^{Glc}-α-Glc*p*-lactose and 2^{1}-α-Glc*p*-cellobiose showed an abundance of cross-ring cleavage ions (loss of 60 Da + H_2O) that have also been found to be characteristic of linear (1→4)-linked oligosaccharides (Maina et al., 2013). On the contrary, in positive ionization spectra, the cross-ring ions characteristic of (1→4)-linkage (loss of 60 Da) were not observed, indicating that the pathway forming $^{0,4}X_0$ ions did not occur as it did in negative mode. On the other hand, cross-ring cleavage ions are also often absent from nonreducing oligosaccharides (Lee et al., 2012; Zhu & Cole, 2001). Thus, analyzing only positive or negative ionization might lead to incorrect identification of an unknown compound containing a 2,4-*O*-disubstituted reducing end residue. The information obtained on fragmentation pathways will be useful for characterization of other oligosaccharides containing a similar branch structure.

A minor portion of unknown product with the same *m*/*z* as isomelezitose was found. It consisted of two labeled residues next to each other and one unlabeled residue at the reducing end. As lactose was not cleaved during the acceptor reaction with WcE392-rDSR, the only possible structure aligning with this description was one that contained fructose at the reducing end, with two consecutive glucosyl residues attached to it. The other $^{13}C_6^{glu}$-labeled oligosaccharides in the lactose reaction mixture always contained, at least, one unlabeled hexose residue in each DP. No products formed exclusively from labeled units were observed, demonstrating that oligosaccharides containing glucose only were not produced. Oligosaccharides with DP ⩾ 5 carried one unlabeled residue, indicating the elongation of fructose-containing products. Products with two unlabeled residues, pointing to lactose-derived products, were only found in DP3 and DP4. However, this was a minor product in DP4 and a major product in DP3. More studies are needed to elucidate the structures of the unknown trisaccharides and oligosaccharides with DP ⩾ 4.

5. Conclusion

The selectivity and regiospecificity of the dextransucrase from *W. confusa* VTT E-90392 were similar to those of *Lc. mesenteroides* B-512F dextransucrase. In the acceptor reactions with analogs lactose and cellobiose, the *Weissella* dextransucrase catalyzed the attachment of a glucosyl residue via α-(1→2)-linkage to the acceptor's reducing end. The prebiotic potential of the branched trisaccharides that formed was previously demonstrated (García-Cayuela et al., 2014; Ruiz-Matute et al., 2011). Moreover, a side product, isomelezitose (6^{Fru}-α-Glc*p*-sucrose), was identified for the first time in a dextransucrase-catalyzed reaction. The full NMR assignment for 2^{1}-α-Glc*p*-cellobiose and the MS^n characterization of the three trisaccharides are reported for the first time. This study enhances the understanding of the catalytic mechanism of *Weissella* dextransucrases and their usability in synthesizing rare oligosaccharides.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data are available showing the HPAEC–PAD profiles of the reaction mixtures of the selected WcE392-rDSR acceptor reactions, the 2D edited-HSQC, TOCSY, and HMBC NMR spectra and the full ^1H and ^{13}C NMR assignments for products I, II, and III, the positive and negative ESI-MS^n spectra of the unlabeled products I, II, and III, and the MS spectrum of the labeled oligosaccharide mixture from WcE392-rDSR lactose reaction. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.foodchem.2015.05.090.

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