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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.

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