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Separation and characterization of dextran extracted from deteriorated sugarcane

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ABSTRACT

In this research work, dextran was extracted from deteriorated sugarcane by alcohol precipitation and purified by gel filtration chromatography. Total acid hydrolysis and enzymatic degradation were utilized to confirm the purity of separated polysaccharide. Using the more recently available techniques such as (^{1}H,^{13}C) and two-dimensional (COSY and HMQC) NMR spectral analysis, methylation GC–MS and MALDI-TOF mass spectrometry, the structure of sugarcane dextran (SC-Dex) was investigated. On the basis of all spectra, SC-Dex showed a branched polysaccharide that contained only D-glucose residues in consecutive α-(1–6) linkages in the main chain with α-(1–3) branches. Methylation analysis showed that, the degree of α-(1–3) branching levels was 4.37%. Several structural fragments were identified from MALDI-TOF spectrum with peak-to-peak mass difference of 162 g mol^{-1}, which confirmed that the repeat unit in SC-Dex was D-glucose. The surface morphology of SC-Dex, revealed the spherically shaped and porous structure. Using HPSEC–MALLS–RI system, the average molecular weight of SC-Dex was estimated to be 1.753×10^6 g mol^{-1} with an index of polydispersity value of 1.069.

1. Introduction

Dextrans are water-soluble polysaccharides, which consist mainly of α-(1–6) linked D-glucopyranose residues with a low percentage of α-(1–2), α-(1–3), and α-(1–4) linked side chains [1]. A polysaccharide usually referred to as dextran compound widely occurs in deteriorated sugarcane and beet. These molecules are derived from the metabolic activities of microorganisms growing during plant cultivation or at some stage during the subsequent processing [2]. Dextran is produced by the dextransucrase enzyme which is secreted by bacteria of the genera *Lactobacillus*, *Leuconostoc* and *Streptococcus*, of which the most commonly found in deteriorated or sour sugarcane, is *Leuconostoc mesenteroides*. Different types of dextrans of varying size and structure are synthesized depending on the dextransucrase produced by the strain [3]. Since the early years of sugar manufacturing, there have been many published scientific reports, on the formation of dextran in sugar factories and refineries. The presence of dextran in the sugar factories leads to a false high polarization, increased viscosity, slow filtration process, lower evaporation rates and elongated crystals [4]. Furthermore, recent research suggests that the endo-dextranase-resistant dextran may have contributed to the hard-to-boil massecuites phenomenon [5].

Walker [6] reported that cane dextrans have similar structure to the dextrans produced by the *L. mesenteroides* strain NRRL B-512, which are predominantly linear, 95% α-(1-6) linkages with 5% branching probably α-(1-3) and have a high polydispersity. Dextran from *L. mesenteroides* B512F contains 95% α-(1-6) linkages and 5% α-(1-3) branch linkages, whereas, insoluble dextran from *L. mesenteroides* 1299 contains 63% α-(1-6), 27% α-(1-2) and 8% α-(1-3) linkages [7].

Dextran is widely applied in pharmaceutical, food, and chemical industries. In pharmaceutical industry, dextran is used as a drug (blood plasma volume expander) whereas in food systems, it is used as adjuvant, emulsifier, carrier, stabilizer and thickener especially in jams and ice creams [8,9]. Dextran hydrogels have immense applications in various biomedical fields including contact lenses, cell encapsulation, drug delivery, and tissue engineering. [10]. Cross-linked dextran known as sephadex is widely used for separation and purification of proteins [9].

The main focus of this research, was to extract dextran from sugarcane juice. Using more recently available techniques such as methylation and GC–MS analysis, 2D NMR spectroscopy including homonuclear ^{1}H/^{1}H correlation spectroscopy (COSY), and heteronuclear ^{13}C/^{1}H multiple quantum coherence experiments

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(HMQC) and matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry, the structure of sugarcane dextran was then revised.

2. Experimental details

2.1. Materials

Sugarcane was obtained from the local market during the season from 2011 to 2012 (Wuxi, China). Dextran T-$_{2000}$ (from *L. mesenteroides*; M_w∼2000 kDa, according to Fluka's specification), dextranase produced by *Chaetomium erraticum*, α-amylase and 2,5-dihydroxybenzoic acid (DHB) were purchased from Sigma–Aldrich (Shanghai, China). All other chemicals and solvents used were of analytical grade.

2.2. Isolation and identification of Leuconostoc strain

Bacterial culture was isolated from sugarcane and raw juice using enrichment media technique. Samples (10 g) were taken and diluted using sterilized distilled water to 100 g. Sample was inoculated in a broth medium containing (w/v): 5% sucrose; 1% tryptone; 0.1% yeast extract; 0.25% K_2HPO_4. The medium was adjusted to pH 5.5 and sterilized in LDZX-50KB autoclave (Shanghai Shen An Medical Instrument Factory, China) at 121 °C for 20 min. After autoclaving, 0.005% sodium azide was added aseptically to the medium for selective isolation of dextran producing *Leuconostocs* sp. Inoculated broth was incubated for 24 h at 25 °C and streaked on the agar plates containing the above medium to isolate pure culture [11]. Bacterial strain was identified according to the Bergey's Manual of Determinative Bacteriology [12] and L/D-lactic acid test [13].

2.3. Precipitation of polysaccharides

sugarcane (500 g) was first cut into small pieces and then 250 mL of 0.005% sodium azide solution was added for selective the dextran producing *Leuconostocs* sp. [11]. After 20 min, the Sugarcane pieces were separated from the sodium azide solution by vacuum filtration. Sugarcane was kept in plastic bags and allowed to deteriorate at 30 °C for 72 h, then the juice extracted using laboratory core press. Sugarcane juice (100 mL) was pipetted into a conical flask and 0.3 mL amylase was added at 55 °C and allowed to stand for 15 min for starch degradation. Trichloroacetic acid solution (6 ml, 10%) was added to remove the protein. The mixture was filtered by using a 0.45 μm filter membrane. Crude polysaccharide was precipitated from the filtered solution using an equal volume of chilled 95% ethanol, shaken vigorously, centrifuged at 10,000 rpm for 15 min at 4 °C and the supernatant decanted. This step was repeated thrice.

2.4. Separation and purification of dextran

Dextran in the precipitated polysaccharide was initially separated by gel filtration using Sephacryl S-500 HR column (1.6 cm × 60 cm) eluted with 50 mmol L^{-1} phosphate buffer (pH 6.5). Every 5 mL of the eluate was collected. All fractions were subjected to the total acid hydrolysis followed by monosaccharide composition analysis, furthermore, the dextran concentration in all fractions was determined based on ICUMSA GS1–15 [1994] and rapid haze method [14], using dextran T-$_{2000}$ as a standard. The peak with the highest dextran content was collected. 1 ml of concentrated polysaccharide solution collected from the peak with the highest dextran content was loaded onto Superdex 200 PG column (1.6 cm × 60 cm, GE Healthcare life science, China), for further purification. Polysaccharides were eluted with 50 mmol L^{-1} phosphate buffer (pH 6.5) containing 100 mmol L^{-1} NaCl at a flow rate of 0.5 mL min^{-1}. Every 5 mL of the eluate was collected. All fractions were subjected to total acid hydrolysis followed by monosaccharide composition analysis.

2.5. Enzymatic hydrolysis of purified polysaccharide

All the fractions collected by Superdex 200 PG column were subjected to enzymatic hydrolysis using dextranase enzyme from *Chaetomium erraticum* (50 mg/mL). Reaction mixture consisted of 2 mL of purified polysaccharide solution (100 mg/mL) mixed with 1 mL of enzyme solution in 20 mM pH 5.4 sodium acetate buffer, was then incubated at 50 °C for 40 min. The hydrolysis product was filtered through a 0.45 μm poresize filter. The hydrolysis product analysis was performed using high performance liquid chromatography (HPLC) and differential refraction index detection (RI-150, Japan). Thermo Aps-2 Hypersil column (250 mm × 4.6 mm ID; Waters, USA) was used for separation. The column thermostat was set at 35 °C. The mobile phase used was acetonitrile: water (70:30). Glucose and isomaltose (Sigma, China) were used as standards.

2.6. Total acid hydrolysis and monosaccharide composition analysis

Monosaccharide composition analysis was conducted using the method described by Mopper et al. [15], with some modification. Sample was hydrolyzed in 1 M H_2SO_4 at 100 °C for 2 h and diluted 50 times. The diluted samples were passed through a 0.45 μm filter and injected into the high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) (ICS-5000, Dionex Corporation, USA), equipped with a CarboPac™ PA20column (250-4 mm I.D., Dionex Corporation, USA) and a guard column (3-25 mm, Dionex Corporation, Canada). Separation was achieved with isocratic eluent (250 mM NaOH) at a flow rate of 0.5 mL/min. A post-column delivery solvent system of 5 and 20% 1 M NaAc was added to the HPAEC-PAD system after 21.1 and 30 min, respectively, at a flow rate of 0.5 mL/min.

2.7. Structural analysis

2.7.1. Fourier transform infrared spectroscopy (FT-IR)

Information about the presence of specific functional groups in isolated SC-Dex were recorded on a Nicolet Nexus 470 FT-IR spectrometer (Spectrum One, Perkin Elmer Co., USA) in the range of 4000–400 cm^{-1} using the KBr-disk method. A total of 32 scans were performed at a resolution of 4 cm^{-1} and 25 °C.

2.7.2. Nuclear magnetic resonance (NMR) study

All NMR spectra were acquired on a Bruker DMX 500 MHz spectrometer (Germany). Sample (25 mg) was dissolved in deuterium oxide (D_2O) at 90 °C for 3 h before NMR analysis. Tetramethyl silane (TMS) was used as an internal reference. The spectra for ^1H, ^{13}C, homonuclear $^1H/^1H$ correlation experiments (COSY), and Heteronuclear Multiple-Quantum Correlation (HMQC) were measured using a standard Bruker pulse sequence. The experiments were conducted at 35 °C. The spectra were analyzed with iNMR software, version 2.6.4.

2.7.3. Methylation and GC–MS analysis

Uronic acids were reduced to neutral polysaccharide before methylation following the previous procedure [16], with a minor modification. Duplicated samples of SC-Dex (5 mg) were dissolved in D_2O (2 mL). To the solution, 50 mg of 1-cyclohexyl-3-(2-morpholinoethyl)-carboiimidemethyl-p-toluenesulfonate was added while using 0.1 mol/L HCl in D_2O to keep pH at 4.75. The solution was left for 1 h under stirring, followed by addition of 5 mL of