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Isolation and characterization of dextran produced by *Leuconostoc citreum* NM105 from manchurian sauerkraut

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ABSTRACT

A water-soluble exopolysaccharide (EPS) was produced by *Leuconostoc ctireum* NM105 from homemade manchurian sauerkraut. After culturing the strain in Man–Rogosa–Sharpe medium containing 5% sucrose at 25 °C for 48 h, the EPS was purified and a yield of 23.5 g/L was achieved. The EPS consisted exclusively of glucose and the weight-average molecular weight was 1.01×10^8 Da. The structural characterization of the purified EPS determined by FT-IR, ^{1}H, ^{13}C and two-dimensional NMR spectroscopy demonstrated that the glucan contained α-(1 → 6)-linked D-glucopyranose units, 2,6-linked D-glucopyranose units and terminal α-D-glucopyranose units at a ratio of 1:1:1. The microstructure of the dried dextran appeared a sheet-like smooth glittering and highly branched surface. The NM105 dextran showed high water solubility and excellent water retention. All the results suggested that the highly α-(1 → 2) branched dextran might have the potential to serve as valuable polymers applied in foods, cosmetics and other fields.

1. Introduction

Polysaccharides are derived from a wide variety of sources: plants (e.g. guar gum, pectin, cellulose, starch), algae (e.g. carrageenan, alginate, agar), bacteria (e.g. xanthan, gellan) and fungus (Donot, Fontana, Baccou, & Schorr-Galindo, 2012). Despite the still domination of the global market for polysaccharides by plants and algae, the microbial productions of EPS have attracted increasing interest in the food and nonfood industries since the strain *Leuconostoc mesenteroides* isolated from wine made the history of bacterial exopolysaccharides. As lactic acid bacteria (LAB) have a GRAS (generally regarded as safe) status, EPS-producing LAB strains can be applied directly to fermented products to modify their properties in the aspects of texture, mouth-feel, taste-perception, rheology and stability (Duboc & Mollet, 2001). The beneficial effects of LAB to the human health have partly attributed to the EPS, e.g. antitumor, antiulcer, antiviral, antimutagenicity, immunomodulatory and antioxidant (Nwodo, Green, & Okoh, 2012). The EPS produced by LAB can be classified into two groups: homopolysaccharides, composed only one type of monosaccharide, and heteropolysaccharides, composed of repeating units that consist two or more different monosaccharides. Based on linkage bonds and nature of monomeric units, the homopolysaccharides can be clustered into four groups: α-D-glucans, β-D-glucans, fructans (e.g. levans, inulin) and others, like polygalactan (Nwodo et al., 2012; Ruas-Madiedo, Hugenholtz, & Zoon, 2002). Depending on the main chain glycosidic linkages, the α-D-glucans can be classified as (i) dextran with α-(1 → 6), (ii) mutan with α-(1 → 3), (iii) alternan with α-(1 → 6) and α-(1 → 3), and (iv) reuteran with α-(1 → 6) and α-(1 → 4) (Bejar et al., 2013; Majumder, Singh, & Goyal, 2009). The dextrans, which are mainly produced from *Leuconostoc*, *Lactobacillus*, *Streptococcus*, and *Weissella* species, differ in the type of glycosidic linkage, degree and type of branch, chain length, molecular weight distribution and conformation of polymeric chain

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(Capek et al., 2011; Freitas, Alves, & Reis, 2011). These variations are influenced by producing strains, cultivation conditions and media compositions.

Leuconostoc species are the primary producers of the dextrans that have a great variety of structure and physico-chemical properties. For instance, the dextran produced from *L. mesenteroides* NRRL B-640 contains D-glucose residues in a linear chain with consecutive α-(1 → 6) linkages and exhibits a typical non-Newtonian pseudoplastic behavior (Purama, Goswami, Khan, & Goyal, 2009); a novel water-soluble dextran synthesized by *L. citreum* SK24.002 is mainly composed of α-1,3 and α-1,6 linked D-glucopyranose units with a ratio of 4:5 (Miao et al., 2014). Among different dextrans, the ones produced by *L. mesenteroides* NRRL B-512F and *L. mesenteroides* NRRL B-1299 have been well characterized and classified. The well-known dextran produced by *L. mesenteroides* B-512F is widely used as blood-plasma substitute due to the low antigenicity, high water solubility and high biological stability in the human bloodstream (de Belder, 1996). Dextrans produced by *Leuconostoc* have also been applied in food (e.g. emulsifying and thickening agents) and chemical (e.g. molecular sieve) industries (Aman, Siddiqui, & Qader, 2012; Duboc & Mollet, 2001; Han et al., 2014). The application of dextrans expands to the eye and skin care products for their excellent biocompatibility, moisturizing properties and stability, and to bakery products to improve softness, crumb texture and loaf volume (Maina et al., 2014). As mentioned above, dextrans are commercially important polysaccharides, thus there is a considerable volume of literature devoted to selecting a highly potent culture capable of producing different types of dextrans for multipurpose uses. However, the existing researches on dextrans have mainly focused on exploring the linear dextrans with low molecular weight and high proportion of α-(1 → 6) glycosidic linkages. Thus far, only a few studies have investigated highly branched dextrans produced from *Leuconostoc* species, and there are less studies on dextrans with a high percentage of α-(1 → 2) linkages. In the present study, an EPS-producing bacterial strain was isolated from homemade manchurian sauerkraut and identified as *L. citreum*. A water-soluble EPS was purified from its culture solution, and the structural characteristics and other physico-chemical properties of the EPS were investigated.

2. Material and methods

2.1. Strain screening and identification

The microorganism NM105 used in this study was isolated from homemade manchurian sauerkraut. *L. citreum* NM105 strain was routinely propagated in MRS medium (20 g/L glucose, 1 mL/L Tween 80, 2 g/L K_2HPO_4, 10 g/L tryptone, 5 g/L yeast extract, 10 g/L beef extract, 5 g/L anhydrous sodium acetate, 2 g/L ammonium citrate, 0.58 g/L $MgSO_4{\cdot}7H_2O$, 0.25 g/L $MnSO_4{\cdot}H_2O$) at 30 °C for 20 h and used to produce exopolysaccharides in MRS medium containing 50 g/L sucrose at 25 °C for 48 h. The NM105 strain was identified through morphological, physico-chemical tests and 16S ribosomal DNA sequence. Genomic DNA was extracted from freshly cultured strains. The 16S rDNA sequence was amplified using universal primers 8F: 5′-AGAGTTTGATCATGGCTCAG-3′ and 1492R: 5′-ACGGTTACCTTGTTACGACTT-3′ (Maiwald, Kappe, & Sonntag, 1994). Conducting the amplification was in a PCR thermal cycler (MyCycler; Bio-Rad Laboratories Inc., USA) by polymerase chain reaction. The PCR reaction factor: 95 °C, 3 min; 95 °C, 30 s, 55 °C, 60 s, 72 °C, 90 s, 30 cycle; 72 °C, 5 min; 4 °C termination reaction. According to the GenBank database, the accurate identification was performed through analysing the 16S rDNA sequence.

2.2. Production and purification of the EPS

The NM105 strain was cultured in MRS medium with 50 g/L sucrose in Erlenmeyer flasks of 500 mL on a rotary shaker (80 rpm) at 25 °C for 48 h, which was inoculated at the amount of 1.5%. EPS was isolated and purified by Kim's method (Kim, Seo, Hwang, Lee, & Park, 2008) with modification. Briefly, the strain cells were removed after centrifugation at 4 °C and 4200 × *g* for 60 min. Three volumes of 95% cold ethanol were added to the supernatant and then incubated overnight at 4 °C. The precipitant (EPS) was gained through centrifugation at 4 °C and 12,000 × *g* for 40 min which then suspended in 250 mL pure water at 30–40 °C. Equal volume of 10% trichloroacetic acid (250 mL) was added and incubated at 4 °C for 10 h, with that centrifuged at 4 °C, 12,000 × *g* for 40 min. Three volumes of 95% cold ethanol were mixed with the supernatant and incubated overnight at 4 °C. After centrifuging, the EPS was resuspended in pure water (~200 mL). A dialysis bag (MW cut-off 14,000) was used to remove the contaminants at 4 °C for 2 days. Further purification was performed by gel-filtration chromatography with a 1.6 cm × 50 cm Sephadex G-100 column. Pure water was used as elution buffer at a flow rate of 0.2 mL/min. Collections were monitored for A220 nm using a spectrophotometer (HD-3 Ultraviolet Detector, Shanghai, China). Finally, the purified EPS was lyophilized and yielded.

2.3. Monosaccharide composition analysis

Analysis of monosaccharide composition was conducted via gas chromatography (GC). The purified EPS (10 mg) was hydrolyzed with 2 M trifluoroacetic acid (TFA, 2 mL) at 120 °C for 6 h and the hydrolysate was evaporated to remove the remanent TFA. Then the dried hydrolysate was converted into acetylated derivatives according to the method of Zhang, Tang, Wang, Zhang, and Zhang (2013). The derivative was subjected to GC analysis by an Agilent 6820 (USA) with a flame ionization detector (FID) and a HP-5 capillary column (30 m × 0.32 mm; i.d 0.25 μm). The operating conditions were as follows: injection volume 3 μl; split ratio 3:1; detector temperature 250 °C; oven temperature 150 °C for 1 min, then 10 °C/min to 200 °C, 200 °C for 10 min, 5 °C/min to 220 °C, 220 °C for 5 min, 1.5 °C/min to 240 °C, 240 °C for 20 min. Standard glucose, arabinose, fructose, rhamnose, galactose and mannose were prepared for comparison. Sugar identification was done through comparing the retention time of the standard samples and the EPS sample.

2.4. Determination of molecular weight

The molecular weight of the EPS was determined by high-performance size-exclusion chromatography (HPSEC, Shimadzu, Japan) with a refractive index detector (RID-10A, Shimadzu, Japan). The sample (2 mg/mL) was filtered through 0.22-μm filter (Sartorius, USA) before injection. A Shodex OH-pak SB-806 column (8.0 mm × 300 mm, Japan) following an OH-pak SB-G guard column was used at 25 °C and pure water was used as the mobile phase at a flow rate of 0.8 mL/min. The injection volume was 20 μl. T-series dextran standards were used to obtain the standard curve and the molecular mass of the sample was extrapolated from the standard curve. Data process was performed by Shimadzu LC solution software (Version 1.26 SP1, Japan).

2.5. Ultraviolet (UV) and FT-IR spectrum analysis

UV spectroscopy of the EPS was recorded on a spectrophotometer. The EPS was dissolved in pure water (1 mg/mL) for scanning between 190 and 350 nm.