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Characterization of a dextran produced by *Leuconostoc pseudomesenteroides* XG5 from homemade wine

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ABSTRACT

A water-souble exopolysaccharide (EPS) produced by *Leuconostoc pseudomesenteroides* XG5 from homemade wine was investigated. The EPS yield of 35.5 g/L was achieved at 30 °C for 48 h in De Man-Rogosa-Sharpe (MRS) medium containing 12.5% sucrose. The EPS was a dextran composed exclusively of glucose and the molecular weight was 2.6×10^6 Da. Fourier transform infrared spectra and nuclear magnetic resonance spectra revealed that the EPS was a dextran containing D-glucose residues in a linear chain with consecutive α-(1 → 6) linkages. Scanning electron microscopy of the EPS appeared a highly branched and porous structure. Rheological studies showed that the EPS had higher viscosity in 0.1 M KCl solution, at lower temperature, or at acidic pH. Thermal gravimetric analysis and differential scanning calorimetric indicated that the EPS had excellent thermal stability with a degradation temperature of 313.80 °C and melting point at 274.14 °C. Water solubility index and water holding capacity of XG5 dextran were 90.2% and 412% respectively. The results suggest that *L. pseudomesenteroides* XG5 might be widely used in the production of linear dextran which has potential to serve as natural agent applied in food and other fields.

1. Introduction

Exopolysaccharides (EPSs) are macromolecules consisting of monosaccharide residues of sugar and sugar derivatives, which are produced during the growth of microorganisms such as bacteria, plants, yeasts, fungi, moulds and blue green algae [1,2]. Due to the unique physical, chemical and biological properties, the EPSs from food-grade latic acid bacteria (LAB) with the GRAS (Generally Recognized As Safe) status have attracted an increasing attention. EPS-producing LAB can be used to modify rheology, texture, stability and mouth-feel of the fermented products [3]. The characteristic functional properties of LAB EPSs make them ideal as stabilizing, viscosifying, emulsifying and gelling agents [4]. The LAB EPSs have been used as encapsulating materials, bioabsorbants, bioflocculants, drug delivery agents, heavy metal removing agents, ion exchange resins. In recent years, many researches have showed that some LAB EPSs may have beneficial effects on the human health due to their biological activities including antitumor, antibiofilm, anti-inflammatory, antiulcer, antioxidant, immunomodulatory and immunostimulatory [5,6]. Moreover, EPSs are able to protect the cells against desiccation, toxic metal ions, antibiotic and phage [7,8].

Dextran is a homopolysaccharide composing of D-glucose units, containing α-(1,6) linkages in the main chain and varied percentages of α-(1,2), α-(1,3), α-(1,4) branch linkages [9]. The dextrans are commercially important EPSs, thus a considerable volume of literature devoted to researching them from numerous species of LAB strains, such as *Leuconostoc mesenteroides*, *Leuconostoc citreum*, *Lactobacillus plantarum* and *Weissella confusa* [10–14]. Dextrans are varied in yields, structures and physico-chemical properties due to the producing microorganisms, culture conditions and medium compositions. For instance, the dextran produced by *L. mesenteroides* FT045 B from an alcohol and sugar mill plant containing 97.9% α-(1,6) linkages and 2.1% α-(1,3) branch linkages [15]. *L. citreum* NM105 was able to synthesize a high weight-average molecular weight dextran (1.01×10^8 Da) which contained more α-(1,2) branch linkages (about 32.4%) and exhibited high water solubility and excellent water retention [16]. Two EPSs produced by the native *L. pseudomesenteroides* R2 strain was identified as linear α-(1,6) dextran which indicated high thermal stability and exhibited the pseudoplastic behavior [17]. A novel water-soluble dextran from *L. citreum* SK24.002 was mainly composed of α-(1,6) and α-

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(1,3) linked D-glucopyranose units with a ratio of 5:4 [12]. Besides, *L. mesenteroides* NRRL B-512F could produce a commercial dextran which contains 95% α-(1,6) linkages and 5% α-(1,3) and α-(1,4) branch linkages [18,19].

Leuconostoc species are the primary producers of the dextrans that have multipurpose uses. However, the existing researches on the strains which synthesized dextrans have mainly focused on *L. mesenteroides* and *L. citreum*, there is little information relating to the dextran from *L. pseudomesenteroides* which is relatively unknown and few studies have been reported the dextran with high production. Thus far, the aim of our study was to isolate and characterize the high yield dextran produced by *L. pseudomesenteroides* XG5 from homemade wine. The dextran was characterized by gas chromatography (GC), high-performance size-exclusion chromatography (HPSEC), fourier transform infrared spectra (FT-IR) analysis, nuclear magnetic resonance (NMR), rheological behavior, scanning electron microscopic (SEM), thermal gravimetric analysis (TGA), differential scanning calorimetric (DSC) analysis, water solubility index (WSI) and water holding capacity (WHC) in order to identify its structural and biochemical characterizations and research the potential applications in the food, cosmetic and other fields.

2. Materials and methods

2.1. Materials

All materials and reagents were purchased from Sigma-Aldrich Co. (St. Louis, Mo, USA) unless otherwise specified.

2.2. Microorganism and culture conditions

The strain XG5 isolated from homemade wine was investigated in this study. De Man–Rogosa–Sharpe (MRS) medium (20 g/L glucose, 10 g/L typtone, 10 g/L beef extract, 5 g/L yeast extract, 2 g/L K_2HPO_4, 5 g/L CH_3COONa, 2 g/L ammonium citrate, 0.58 g/L $MgSO_4 \cdot 7H_2O$, 0.25 g/L $MnSO_4 \cdot H_2O$ and 1 mL of Tween 80) was used for the growth of the organism which propagated at 30 °C for 18 h. The strain was cultured to produce EPS in MRS medium containing 12.5% sucrose at 30 °C for 48 h under a static condition.

2.3. Strain identification

Characteristics of strain XG5 were identified by morphological, biochemical and physiological tests, including morphological observation, Gram reaction, gas production and the utilization of sole carbon source. The strain identification was also confirmed by partially sequencing 16S rDNA genes analysis. Genomic DNA was extracted from freshly cultured strains. The bacterial universal primers with the following sequencing: 8F (5-AGAGTTTGATCATGGCTCAG-3) and 1492R (5-ACGGTTACCTTGTTACGACTT-3) were used to amplify the 16S rDNA genes of target isolate. The polymerase chain reaction (PCR) mixture (100 μL) composed of DNA template (4 μL), each primer (4 μL), 2 × mix (4 μL) and dd H_2O (88 μL). PCR reaction was conducted as follows: initial denaturation at 95 °C for 3 min; 30 cycles consisting of denaturation at 95 °C for 30 s, annealing at 55 °C for 60 s and extension at 72 °C for 5 min; a final extension step at 72 °C for 5 min. The 16S rDNA was sequenced and compared with GenBank database.

2.4. Isolation and purification of the EPS

The XG5 strain was cultivated in MRS medium supplemented with 12.5% sucrose at 30 °C for 48 h under a static condition. After incubation, the isolation and purification of EPS were performed by a modified method of Yang et al. [16]. Briefly, the fermentation broth was heated at 100 °C for 15 min to inactivate enzymes capable of degrading the EPS [20]. Then the culture was centrifuged at 4 °C and 12,000*g* for 40 min to eliminate the strain cells. The EPS was precipitated by adding three fold volumes of 95% (v/v) cold ethanol to the cell-free culture and kept it for overnight at 4 °C. Then crude EPS was collected by centrifugation (12,000*g*, 30 min) and the EPS pellet was dissolved in deionized water. Trichloroacetic acid (10%, w/v) was added to the EPS solution to give the final concentration of 5% and then left overnight at 4 °C. Centrifugation (12,000 × g for 40 min at 4 °C) was carried out to remove the precipitated proteins. The supernatant was treated with three fold volumes of 95% cold ethanol and left it for overnight (4 °C) to precipitate the EPS which was gained through centrifugation (12,000*g* for 30 min at 4 °C). The precipitated EPS was dissolved in deionized water and dialyzed (MWCO 14,000 Da) with deionized water at 4 °C for 48 h. The further purification of the crude bacterial EPS solution was subjected by gel-filtration chromatography using a Sephadex G-100 column (1.6 cm × 50 cm, GE Healthcare, Fairfield, CT, USA). The eluted solution was deionized water with a flow rate of 0.5 mL/min. The purified EPS solution was pooled, concentrated, lyophilized and yielded.

2.5. Monosaccharide composition analysis

The purified EPS (10 mg) was hydrolyzed with 2 mL trifluoroacetic acid (TFA) (2 mol/L) at 120 °C for 4 h to completely release the monosaccharide. The remanent TFA was eliminated via evaporating the hydrolyzed solution. After that, the acetylated derivative of dried hydrolyzate was achieved as previously described [21]. The derivatives of standard sugars (glucose, mannose, L-arabinose, lactose, galactose) were prepared in the same way. The GC analysis of derivative was conducted on Agilent 6820 (Agilent Technologies, Palo Alto, CA, USA) equipped with a flame ionization detector (FID) and a HP-5 fused silica capillary column (30 m × 0.32 mm × 0.25 μm, Agilent Technologies, Palo Alto, CA, USA). The following operating conditions were used as below: the injector and detector temperature were all set at 250 °C; the injection volume was 3 μL; the initial column temperature was held at 200 °C for 10 min, then programmed at a rate of 5 °C/min to 220 °C for 5 min and increased to 240 °C for 20 min with a rate of 1.5 °C/min.

2.6. Molecular weight analysis

Molecular weight of the EPS was determined through HPSEC quipped with the Optilab T-rEX refractive index detector (RI, Wyatt Technology, Santa Barbara, CA, USA) and a Shodex OH-pak SB-806 HQ column (8.0 mm × 300 mm, Showa Denko K.K., Tokyo, Japan), at an internal temperature of 25 °C. The EPS solution (2 mg/mL) was filtered by the 0.22 μm cellulose acetate filters (filter, Sartorius, Germany) before injection, and the injection volume was 20 μL. The column was eluted with eionized water at a flow rate of 0.8 mL/min. Different molecular weight of dextrans (1170, 1740, 2400, 3755 kDa) were carried out as the standards for the determination of EPS molecular weight. The calibration curve of standard dextrans was used to calculate the molecular weight. Date processing was performed with Shimadzu Liquid Chromatography solution software (Version 1.26 SP1, Shimadzu, Japan).

2.7. UV and FT-IR spectrum analysis

UV spectroscopy analysis of the purified EPS was performed on a Shimadzu spectrophotometer (UV-1603, Kyoto, Japan). The spectrum of EPS solution (1 mg/mL) was recorded between 190 and 350 nm. The UV spectroscopy was used to check the purity of the purified EPS.

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