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Structural analysis and characterization of dextran produced by wild and mutant strains of *Leuconostoc mesenteroides*

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ABSTRACT

An exopolysaccharide known as dextran was produced by *Leuconostoc mesenteroides* KIBGE-IB22 (wild) and *L. mesenteroides* KIBGE-IB22M20 (mutant). The structure was characterized using FTIR, ^{1}H NMR, ^{13}C NMR and 2D NMR spectroscopic techniques, whereas surface morphology was analyzed using SEM. A clear difference in the spectral chemical shift patterns was observed in both samples. All the spectral data indicated that the exopolysaccharide produced by KIBGE-IB22 is a mixture of two biopolymers. One was dextran in α-(1 → 6) configuration with a small proportion of α-(1 → 3) branching and the other was levan containing β-(2 → 6) fructan fructofuranosyl linkages. However, remarkably the mutant only produced dextran without any concomitant production of levan. Study suggested that the property of KIBGE-IB22M20, regarding improved production of high molecular weight dextran in a shorter period of fermentation time without any contamination of other exopolysaccharide, could be employed to make the downstream process more feasible and cost effective on large scale.

1. Introduction

Dextran is a biopolymer with several known applications and it is produced by a variety of lactic acid bacteria. This bacterial exopolysaccharide (EPS) has several advantages over other polysaccharides produced by plants, seaweeds and animals due to its biodegradability and biocompatibility properties. Dextran produced by different strains also varies in their glycosidic linkages, degree and/or type of branching, molecular mass, physical and chemical characteristics (Maina, Tenkanen, Maaheimo, Juvonen, & Virkki, 2008). *Leuconostoc mesenteroides* NRRL B-512F is used for the commercial production of water soluble dextran which contains predominantly 95% of linear α-(1 → 6) linkages as main backbone and 5% of α-(1 → 3) branch linkage (Kim & Robyt, 1995; Vettori, Franchetti, & Contiero, 2012). Due to the low degree of antigenicity and high percentage of α-(1 → 6) glycosidic linkage this dextran is used for clinical purpose. The structure of dextran can be linear or branched depending on the producing strains and several stains. Several strains have been previously reported which can produce a number of types of dextran with different degree and types of branching. Characterization of dextran is an important factor for its utilization and several workers characterized dextran using well known advance techniques such as one dimensional (1D) or two dimensional (2D) nuclear magnetic resonance spectroscopy (NMR), Fourier-transform infrared (FTIR) and scanning electron microscopy (SEM) (Bounaix et al., 2009; Maina et al., 2008; Patel, Kothari, Shukla, Das, & Goyal, 2011; Purama, Goswami, Khan, & Goyal, 2009; Shingel, 2002; Van Leeuwen, Leeflang, Gerwig, & Kamerling, 2008; Wang, Deng, Li, & Tan, 2007). Several effective techniques such as physical, chemical and site directed mutagenesis used to improve the production of dextran which would ultimately lead to the production of biopolymer on industrial scale level (Kitaoka & Robyt, 1998; Patel & Goyal, 2010; Smith & Zahnley, 1997).

In order to develop a sustainable mutant for industrial production of dextran, a random approach was undertaken to improve the production of dextransucrase which is responsible for the production of dextran, by exposing the parent strain (*L. mesenteroides* KIBGE-IB22) to UV irradiation for different time intervals (Siddiqui, Aman, & Qader, 2013). A promising mutant (*L. mesenteroides*

Abbreviations: EPS, exopolysaccharide; FTIR, Fourier-transform infrared; SEM, scanning electron microscopy; KIBGE-IB22, wild type; KIBGE-IB22M20, mutant; ^{1}H NMR, proton nuclear magnetic resonance spectroscopy; ^{13}C NMR, carbon nuclear magnetic resonance spectroscopy; 2D NMR, two-dimensional nuclear magnetic resonance spectroscopy; COSY, homonuclear correlation spectroscopy; DQF-COSY, double quantum-filtered phase-sensitive COSY; TOCSY, total correlation spectroscopy; HSQC, heteronuclear single-quantum correlation spectroscopy; HMBC, heteronuclear multiple-bond correlation spectroscopy; NOESY, nuclear Overhauser effect spectroscopy; ROESY, rotating frame nuclear Overhauser effect spectroscopy.

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KIBGE-IB22M20) was developed that showed over expression of dextransucrase as compared to the wild type. In the current study dextran produced by wild and mutant strains were characterized and structural characteristics including average molecular weight and fermentation parameters of dextran production were studied in detail.

2. Material and methods

2.1. Bacterial strains

The strains used for the production of dextran in this study were *L. mesenteroides* KIBGE-IB22 (wild type) [GenBank: JQ658345] and its UV generated mutant *L. mesenteroides* KIBGE-IB22M20 [GenBank: JQ658346]. Wild strain was isolated from an indigenous source and the method of generating its mutant is discussed previously (Siddiqui et al., 2013). The isolates were stored in 15.0% glycerol at −80 °C.

2.2. Production and purification of dextran

Dextran was produced by batch fermentation in a pre-defined medium (pH 7.5) and the culture was incubated at 25 °C for 18 h under static condition (Aman, Siddiqui, & Qader, 2012). After fermentation, dextran was precipitated and purified as described previously (Sarwat, Qader, Aman, & Ahmed, 2008).

2.3. Optimization of fermentation parameters

Various physical and chemical factors such as fermentation time, substrate concentration, medium pH and temperature for the production of dextran from both wild and mutant strains of *L. mesenteroides* were altered by using one variable at a time methodology. Initially, culture media flasks were incubated for different time intervals (6–36 h) at 25 °C for the maximum production of dextran. Different sucrose concentration ranging from 2.0% to 30.0% was incorporated in the basal medium keeping the other parameters constant as a carbon source for maximum dextran production. Similarly different media pH ranging from 5.0 to 10.0 (±0.1) and temperature from 10 °C to 40 °C (±1 °C) were used for maximum dextran production for both wild and mutant strains.

2.4. Determination of average molecular weight of dextran

Average molecular weight of dextran was calculated using gel permeation chromatographic system (Econo pump EP-1, Bio-Rad, USA). Column (46.0 cm × 1.6 cm) containing Sephacryl-500 HR (GE Healthcare Bio-Sciences AB, Sweden) was packed under controlled pressure and pre-equilibrated with citrate buffer (pH 5.0, 0.3 M). Dextran (2.0 mg/mL) was loaded on the column and eluted using the same buffer with a constant flow rate (1.0 mL/min). Different fractions (1.0 mL) were collected using a fraction collector (2110 fraction collector, Bio-Rad, USA). Different standard such as Blue dextran (2,000,000 Da; Sigma, USA) and Industrial dextran (5,000,000–40,000,000 Da; Sigma, USA) were used for the estimation of average molecular weight of dextran produced by wild and mutant strains.

2.5. Physico chemical properties of dextran

For the compositional analysis of dextran produced by wild and mutant strains different physico-chemical properties were studied. The total sugar content was determined using anthrone method (Hassid & Abraham, 1957); whereas Nelson and Somogyi's method was used for reducing sugar (Nelson, 1944; Somogyi, 1945). Total protein content was calculated using bovine serum albumin (BSA) as a standard (Lowry, Rosebrough, Farr, & Randall, 1951). Viscosity of the dextran (5.0% solution) produced by wild and mutant was determined at 24 °C using spindle #01 with a speed of 50 rpm on Brookfield digital viscometer (DV-II). Ash content was analyzed by keeping the dextran in a pre-weighed crucible at 850 °C for 12–14 h, until the weight becomes constant in muffle furnace.

2.6. Scanning electron microscopy (SEM)

Dried dextran sample was bound to SEM stub with the assistance of a double-sided tape and encrusted with gold (Au) targeted up to 300 Å in a Quick Auto Coater (JFC-1500 JEOL, Tokyo, Japan). The coated sample was analyzed using a JSM 6380A scanning electron microscope (JEOL, Tokyo, Japan) operated at different accelerated voltages (10.0, 20.0 and 25.0 kV) and the images of the pattern were observed at different magnification powers.

2.7. Structural analysis of dextran

2.7.1. Fourier-transform infrared (FTIR)

The FTIR spectrum was analyzed using Nicolet Avtar 370 DTGS spectrometer coupled with Smart Omni sampler (Thermo Electron Corporation, USA) and interfaced with EZ-Omnic software for the analysis of dextran. The spectrum was scanned in the wave number range of 500–4000 (cm^{-1}) by accumulating 55 scans with a resolution of 10 cm^{-1}.

2.7.2. NMR spectroscopic analysis

1D and 2D ^1H NMR spectra were recorded in D_2O, at 303 K, at pD 7; on Bruker 600 DRX equipped with a cryo-probe. Spectra were calibrated with internal acetone [δH 2.225, δC 31.45]. Double quantum-filtered phase-sensitive COSY (DQF-COSY) experiments were performed using data sets of 4096 × 256 points. TOCSY experiments were performed with spinlock times of 100 ms, using data sets (t1 × t2) of 4096 × 256 points. In all homo-nuclear experiments, the data matrix was zero-filled in both dimensions to give a matrix of 4K × 2K points and resolution was enhanced in both dimensions by a cosine-bell function before Fourier transformation. Coupling constants were determined by 2D phase sensitive DQF-COSY (Piantini, Sorensen, & Ernst, 1982; Rance et al., 1983). HSQC and HMBC experiments were measured in the ^1H detected mode via single quantum coherence with proton decoupling in the ^{13}C domain, using data sets of 2048 × 256 points. Experiments were carried out in the phase-sensitive mode (States, Haberkorn, & Ruben, 1982). A 60 ms delay was used for the evolution of long-range connectivity in the HMBC experiment. In all hetero-nuclear experiments the data matrix was extended to 2048 × 1024 points using forward linear prediction extrapolation. Data acquired was processed using TopSpin software (version 2.1).

3. Results and discussion

Natural exopolysaccharides with their structural diversity and functional versatility have gained commercial importance in the field of glyco-technology. Most of the polysaccharides used in various industries have been derived from various sources such as animals, plants and seaweeds. With the advancement in fermentation technology various exopolysaccharides of bacterial origin are now produced on commercial scales as compared to synthetic polysaccharides. Among several reported biopolymers, dextran is rated one of important exopolysaccharides which have several renowned applications in different industries depending on its molecular weight. High molecular weight dextran can be hydrolyzed by chemical or enzymatic means into several small fraction of specific molecular mass for specific uses.