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Structural analysis and properties of dextran produced by *Leuconostoc mesenteroides* NRRL B-640

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ABSTRACT

A water-soluble dextran was produced by purified dextransucrase from *Leuconostoc mesenteroides* NRRL B-640. The dextran was purified by alcohol precipitation. The structure of dextran was determined by FT-IR, ^{1}H NMR, ^{13}C NMR and 2-dimensional NMR spectroscopic techniques. NMR techniques (1D ^{1}H, ^{13}C and 2D HMQC) were used to fully assign the ^{1}H and ^{13}C spectra. All the spectral data showed that the dextran contains D-glucose residues in a linear chain with consecutive α(1 → 6) linkages. No branching was observed in the dextran structure. The viscosity of dextran solution decreased with the increase in shear rate exhibiting a typical non-Newtonian pseudoplastic behavior. The surface morphology of dried and powdered dextran studied using Scanning electron microscopy revealed the cubical porous structure.

1. Introduction

Dextrans are polymeric chain of glucosyl units, synthesized by dextransucrase by the transfer of D-glucosyl unit from sucrose to acceptor molecules. This polymerization results in formation of dextran and fructosyl unit is released free. Dextrans feature substantial number of consecutive α(1 → 6)-linkages in their main chain, usually comprising more than 50% of the total linkages. These α-D-glucans also possess side chains, stemming mainly from α(1 → 3) and occasionally from α(1 → 4)- or α(1 → 2)-branched linkages. A survey of 96 strains of dextran-producing bacteria was done to classify dextrans by their structure and properties and identify a suitable strain, for pharmaceutical industry (Jeanes et al., 1954). Different types of dextrans of varying size and structure are synthesized depending on the dextransucrase produced by the strain (Leathers, 2002; Robyt, 1995; Seymour & Knapp, 1980). Most of the recent work with dextrans has been conducted with *Leuconostoc mesenteroides* particularly with the strain NRRL B-512F. The dextran synthesized by this strain is a homopolysaccharide containing 95% α(1 → 6) glucosidic linkages in the main linear chain and remaining 5% of α(1 → 4), α(1 → 3) and a very few α(1 → 2) branched linkages. The branches are mostly 1–2 glucose units long. The solubility of dextran depends upon the branched linkage pattern. Presence of 95% linear linkages makes this dextran water-soluble, which makes it suitable for various applications (Leathers, 2002).

Extensive work has been done on optimization and modification of the fermentation processes for improved production of dextran (Ajongwen & Barker 1993; Lazic, Velzkovic, Vucetic, & Vrvic, 1993). Dextran synthesis takes place outside the cells by dextransucrase in presence of sucrose. Immobilization of whole cell and purified enzyme preparations were reported by several authors for industrial dextran production (El-Sayed, Mahmoud, & Coughlin, 1990; Lebrun, Junter, Jouenne, & Mignot, 1994; Monsan & Lopez 1981). The importance of soluble dextran synthesized by *L. mesenteroides* lies in its wide applications in pharmaceuticals, food, agricultural and fine chemical industries. The soluble dextrans have well-documented wide applications in industry (Naessens, Cerdobbel, Soetaert, & Vandamme 2005; Purama & Goyal, 2005). Dextrans have been successfully used in the transglucosylation reaction of low molecular weight drugs to improve their water solubility (Shavej, Richard, Alistair, & John, 2006). These recent applications need the exploration of highly soluble dextrans producing microorganisms to meet the requirement of industry. In the present study the dextran was synthesized using dextransu-

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crase from *L. mesenteroides* NRRL B-640 and the structure of dextran was determined by FT-IR, NMR spectroscopic techniques and scanning electron microscopy.

2. Materials and methods

2.1. Production of dextran

The dextran was produced by incubating 0.2 ml dextransucrase (0.58 mg/ml protein of specific activity 23 U/mg, purified by 10% PEG-1500 fractionation) in 100 ml of 5% sucrose in 20 mM sodium acetate, pH 5.4, containing 0.3 mM $CaCl_2$ and 15 mM sodium azide at 28 °C for 48 h. After the completion of incubation, the dextran was precipitated using a final concentration of 65% ethanol and re-suspended in water, this step was repeated. The precipitate was heat dissolved in water and dried by lyophilization.

2.2. Determination of dextran concentration

The polysaccharide content was determined by phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) in a micro-titre plate (Fox & Robyt 1991). To 25 µl of sample containing dextran in a microtitre plate, 25 µl of 5% (w/v) phenol was added. The plate was mixed at slow speed on a vortex mixer for 30 s. The plate was then placed onto ice bath and 125 µl of concentrated sulfuric acid was added to each well containing sample and phenol. The plate was again mixed for 30 s incubated in water bath at 80 °C for 30 min and cooled and the absorbance was determined at 490 nm on a microtitre plate reader. Standard curve was prepared using dextran (10 kDa) in the concentration range 0.1–1 mg/ml.

2.3. Optical rotation

The optical rotation for dextran solution in water in a 10-ml quartz cuvette was recorded using a polarimeter (Perkin-Elmer Instruments, Model 343 Polarimeter) using a sodium D-line (589 nm) at 25 °C.

2.4. FT-IR spectrum

The FT-IR spectrum using spectrometer (Perkin-Elmer Instruments, Spectrum One FT-IR Spectrometer) was recorded for purified dextran in a KBr pellet.

2.5. NMR studies

NMR experiments were performed in a Varian, AS400 spectrometer. The dextran was vacuum dried and then exchanged with deuterium by successive lyophilization steps in D_2O (99.6% atom ^2H, Aldrich) and then dissolved in 0.5 ml of D_2O (10 mg/ml). Tetramethyl silane (TMS) was used as an internal reference. 1D ^1H NMR and ^{13}C NMR spectra and 2D ^1H, ^{13}C HMQC spectra of dextran were recorded at a base frequency of 100 MHz for analyzing the linkage composition.

2.6. Rheological analysis of dextran

The steady shear viscosity (η) measurement were recorded for the semi-dilute glucan polymer (0.5%) at 25 °C using a rheometer (Make, Thermo Electron model, Haake rheostress RSI) interfaced with a HAAKE RheoWin 323 software. The shear rate was in the range of 0.1–1000 s^{-1}.

2.7. Scanning electron microscopic analysis of dextran

A sample of the dried polymer dextran was attached to the S.E.M stub with double-sided tape then coated with ~10 nm Au in a sputter coater (SCH 620, Leo). The surface of the sample was viewed in Scanning Electron Microscope (Leo1330 VP) operated at 10.0 kV.

3. Results and discussion

The structure of *L. mesenteroides* NRRL B-640 dextran was characterized using FT-IR, ^1H NMR, ^{13}C NMR and 2-dimensional NMR (HMQC) spectroscopy. Dextran was synthesized using purified dextransucrase.

3.1. Optical rotation and Fourier-transform infrared spectrometric (FT-IR) analysis

The dextran synthesized from dextransucrase of *L.* mesenteroides NRRL B-640 showed an optical rotation $[\alpha]_D^{20}$ of +159° at $C^{0.25}$. The FT-IR spectra were used to investigate the functional groups of commercial dextrans and pullulans and their nature in terms of their monomeric units and their linkages (Shingel, 2002). Several other repots also supported the use of FT-IR spectral data for the characterization of glucans showing anti-cancer properties (Cao et al., 2006; Liu et al., 2007). FT-IR spectrum of the purified dextran is presented in Fig. 1. The band in the region of 3400 cm^{-1} was due to the hydroxyl stretching vibration of the polysaccharide (Liu et al., 2007). The band in the region of 2930 cm^{-1} was due to C–H stretching vibration and the band in the region of 1639 cm^{-1} was due to carboxyl group (Cao et al., 2006; Liu et al., 2007). The absorption peak at 906 cm^{-1} indicates the existence of α-glycosidic bond. The main characteristic bands found in the spectra of dextran at 1154, 1103 and 1020 cm^{-1} are due to valent vibrations of C–O and C–C bonds and deformational vibrations of the CCH, COH and HCO bonds. The band at 1154 cm^{-1} is assigned to valent vibrations of C–O–C bond and glycosidic bridge. The peak at 1103 cm^{-1} is due to the vibration of the C–O bond at the C-4 position of glucose residue (Shingel, 2002). The presence of peak at 1020 cm^{-1} is due to the great chain flexibility present in dextran around the $\alpha(1 \rightarrow 6)$ glycosidic bonds as shown earlier (Shingel, 2002). FT-IR spectral analysis of *L. mesenteroides* NRRL B-640 dextran showed that it contains $\alpha(1 \rightarrow 6)$ linkages. This was further confirmed by ^1H NMR and ^{13}C NMR analysis.

3.2. ^1H NMR analysis of dextran

The anomeric proton resonances for the 400 MHz ^1H NMR spectrum of $\alpha(1 \rightarrow 6)$ dextran are shown in Fig. 2. *L. mesenteroides* NRRL B-640 is shown to produce dextransucrase that gives highly linear and soluble dextran (Seymour, 1979a; Uzochukwu, Balogh, Loefler, & Ngoddy, 2002). Sidebotham (1974) reported that various dextrans have ^1H NMR spectral resonances (C-2, C-3, C-4, C-5 and C-6) in the 3- to 4-ppm region and the hemiacetal C-1 resonance in 4–6 ppm region. Seymour (1979a) showed that the ^1H NMR spectral region for anomeric carbon of dextran from *L. mesenteroides* NRRL B-1355 contained a resonance at 4.95 ppm and the branched linkages contained the resonance peak at 5.3 ppm. Seymour (1979b) observed the distribution of ^1H NMR spectral resonances between 3 and 6 ppm for different dextrans. The resonance at 4.96 ppm is due to the H-1 of the $\alpha(1 \rightarrow 6)$ glucosyl residues of main chain (Seymour, 1979b). The assignments for different resonances of ^1H NMR are presented in Table 1.

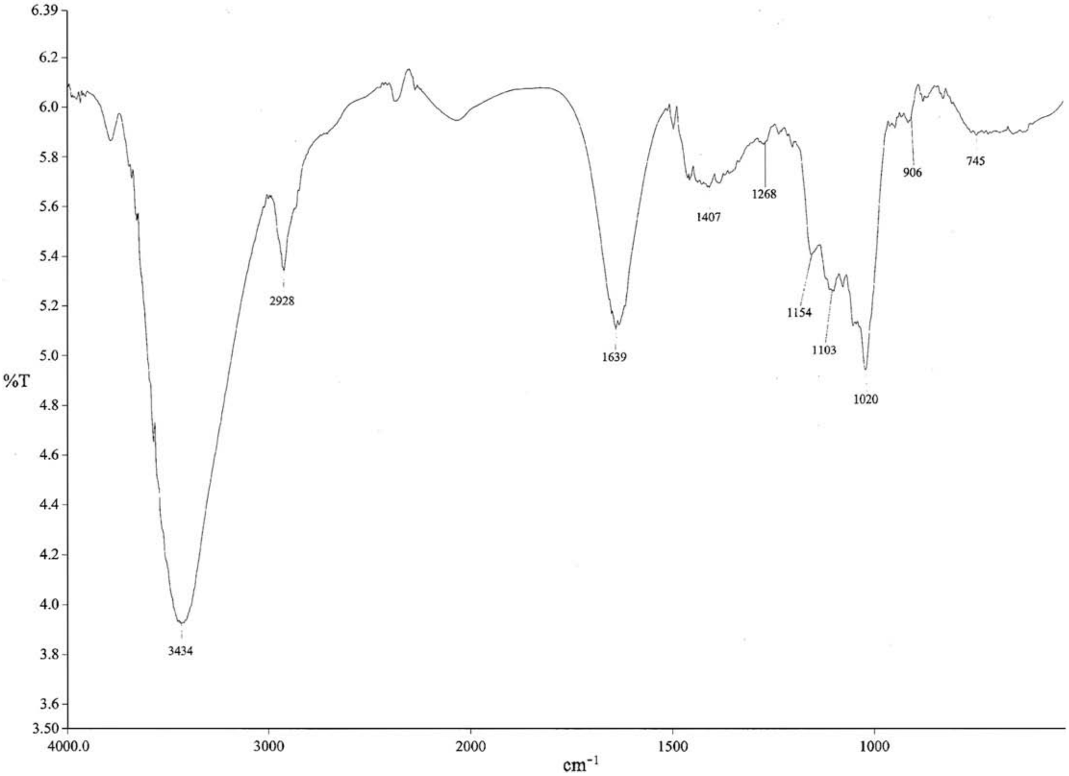

Fig. 1. FT-IR (KBr) spectrum of dextran produced from the purified dextransucrase from *Leuconostoc mesenteroides* NRRL B-640.

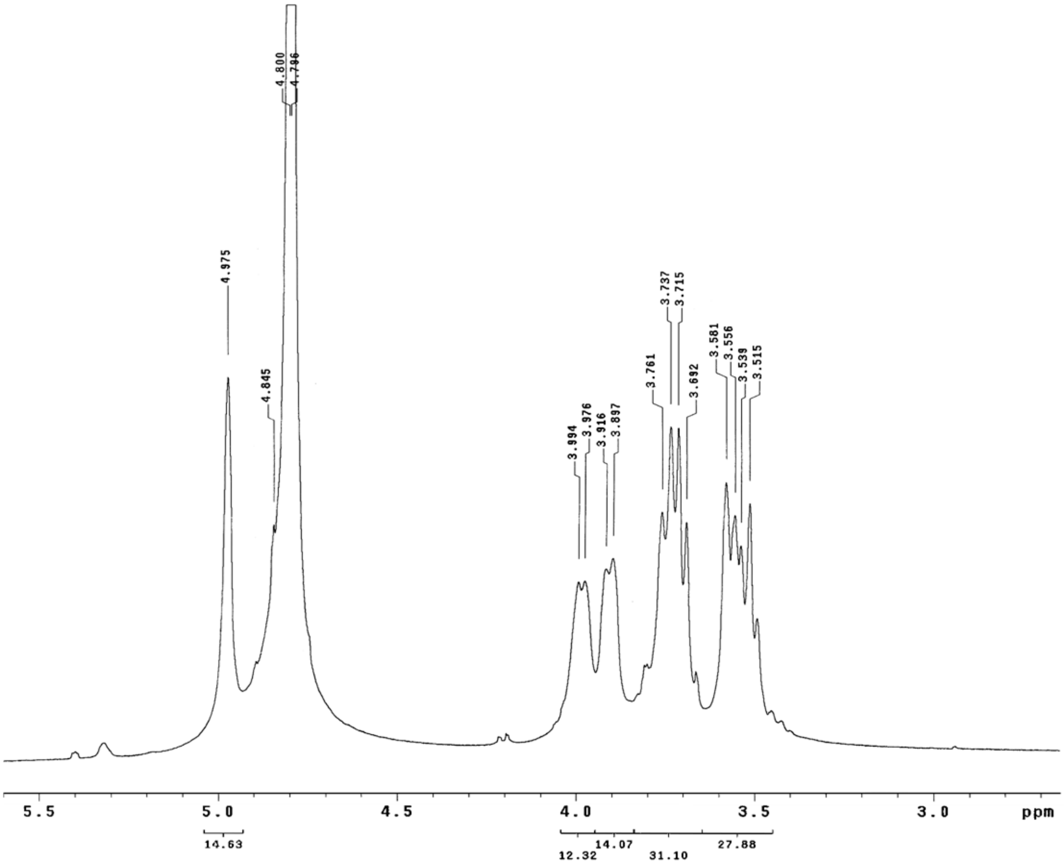

Fig. 2. ^{1}H NMR (400 MHz, D_2O) spectrum of dextran produced from the purified dextransucrase from *Leuconostoc mesenteroides* NRRL B-640.

Table 1
^{1}H and ^{13}C NMR chemical shifts of dextran from *Leuconostoc mesenteroides* NRRL B-640

Atoms	1	2	3	4	5	6
^{13}C	97.81	71.56	73.56	69.68	70.30	65.70
^{1}H	4.98	3.58	3.73	3.54	3.92	3.99
$^{3}J_{H,H}$ (Hz)	–	10	9.6	9.6	7.6	7.2

3.3. ^{13}C NMR analysis of dextran

The dextran showed six prominent ^{13}C NMR resonances at 100 MHz: 97.867, 71.563, 73.563, 69.660, 70.335 and 65.706 ppm (Fig. 3), which is characteristic of linear dextran (Seymour, 1979a; Uzochukwu et al., 2002). Seymour (1979a) employed ^{13}C NMR spectroscopy to examine the structure of a series of dextrans and established that linear dextran has six prominent resonances. The ^{13}C NMR spectra displayed two major regions; (a) the 95- to

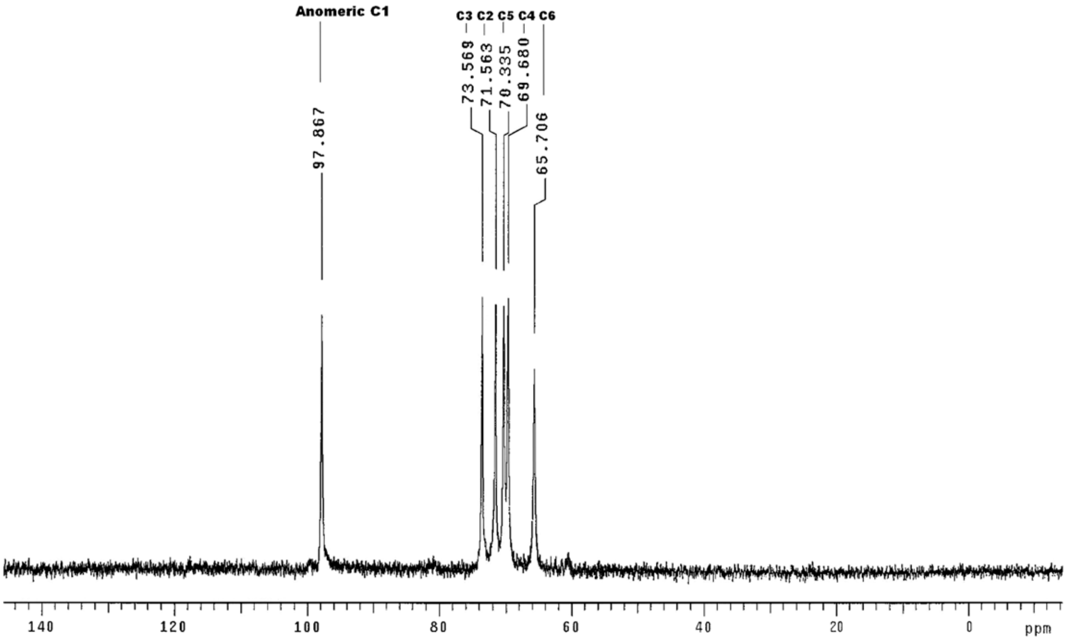

Fig. 3. ^{13}C NMR (100 MHz, D_2O) spectrum of dextran produced from the purified dextransucrase from *Leuconostoc mesenteroides* NRRL B-640.

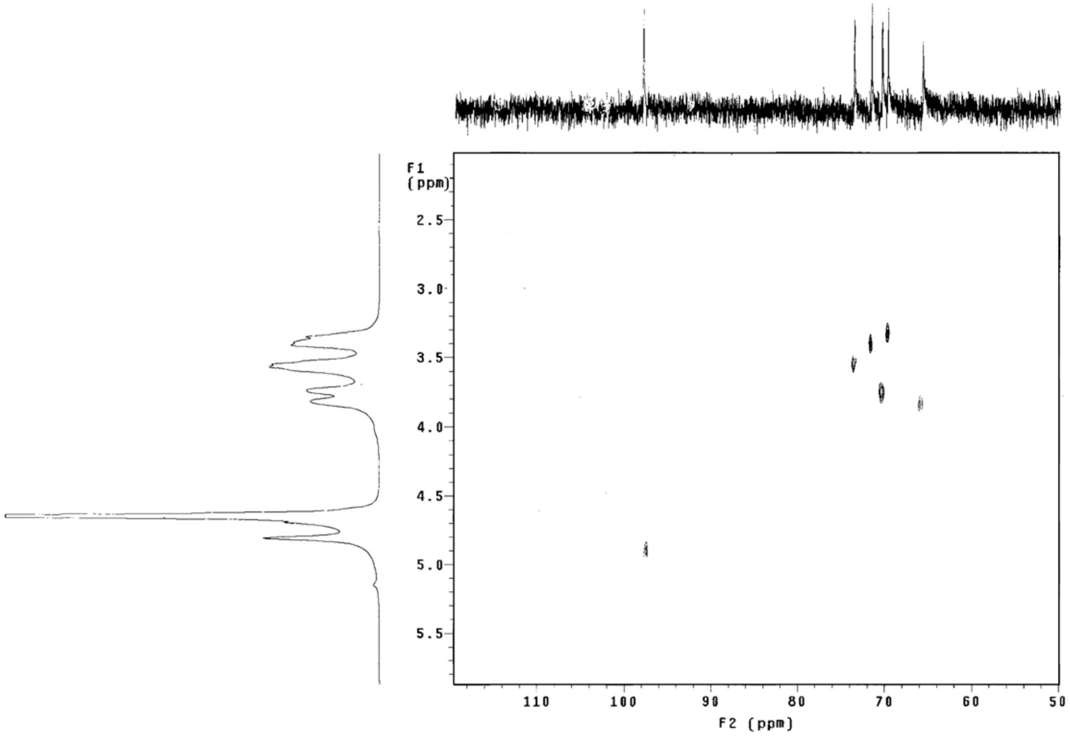

Fig. 4. $^{13}C/^{1}H$ 2D HMQC NMR spectrum of dextran in D_2O.

105-ppm region, which is the anomeric region and (b) the 75- to 85-ppm region for dextrans branched at C-2, C-3, or C-4. ^{13}C NMR resonances with in the 70- to 75-ppm region are associated with free positions at C-2, C-3 and C-4 residues. No additional peaks were observed in the region of 75–85 ppm indicating that the absence of branched linkages (Seymour, 1979a). The major resonance in the anomeric region occurs at 98.7 (97.46) ppm showing that the C-1 is linked. An equally intense signal at 66.5 (65.30 ppm), indicates that most of the C-6 are also linked (Uzochukwu et al., 2002). The equal peak intensities at 97.86 and 65.7 confirmed that the glucose residues in dextran are linked by $\alpha(1 \rightarrow 6)$ glycosidic bond and no additional peaks 75–85 ppm confirmed that the dextran synthesized by the purified dextransucrase from *L. mesenteroides* NRRL B-640 is a highly linear dextran with $\alpha(1 \rightarrow 6)$ glycosidic bonds. The assignments for different resonances of ^{13}C NMR are presented in Table 1.

3.4. Two-dimensional NMR analysis of dextran

It is possible to correlate each ^{1}H with its directly attached ^{13}C using Heteronuclear Multiple Quantum Correlation (HMQC) technique (Ascêncio, Orsato, França, Duarte, & Noseda, 2006). HMQC correlations between ^{1}H and their corresponding ^{13}C of dextran from *L. mesenteroides* NRRL B-640 showed six correlations (Fig. 4). The correlation values given in Table 1 also confirmed that the dextransucrase elaborated by this microorganism synthesizes only a linear dextran with $\alpha(1 \rightarrow 6)$ glycosidic bonds.

3.5. Rheological properties of dextran

The steady shear measurements for the semi-dilute dextran solution showed that the viscosity decreased with the increase in shear rate and exhibited a typical non-Newtonian pseudoplastic behavior (Fig. 5). A similar pseudoplastic behavior of viscosity was observed in case of exopolysaccharide, EPS-WN9 from *Paenibacillus* sp. WN9 (Seo et al., 1999) and EPS450 from *Bacillus* sp. I-450 (Kumar, Joo, Choi, Koo, & Chang, 2004). The exponential decrease in viscosity with increase in shear rate (shear-thinning effect) was also noticed in case of rhamsam produced by *Alcaligenes* sp. which had the largest side chain, while gellan produced by *Psuedomonas elodea* exhibited least shear-thinning, since this polysaccharide had no side chain in the backbone structure (Kwon, Foss, & Rha, 1987).

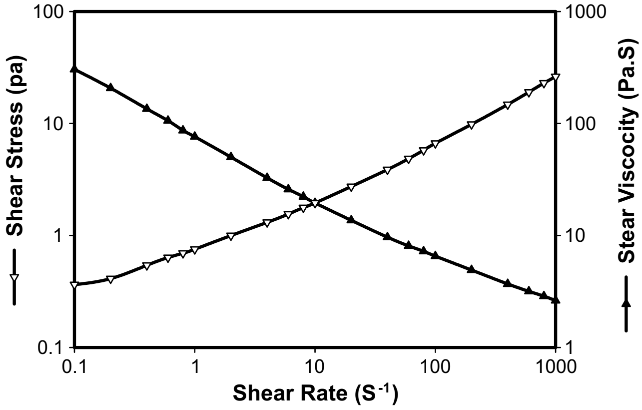

Fig. 5. Viscosity–shear rate profile of glucan at 0.5 g/l measured at 25 °C.

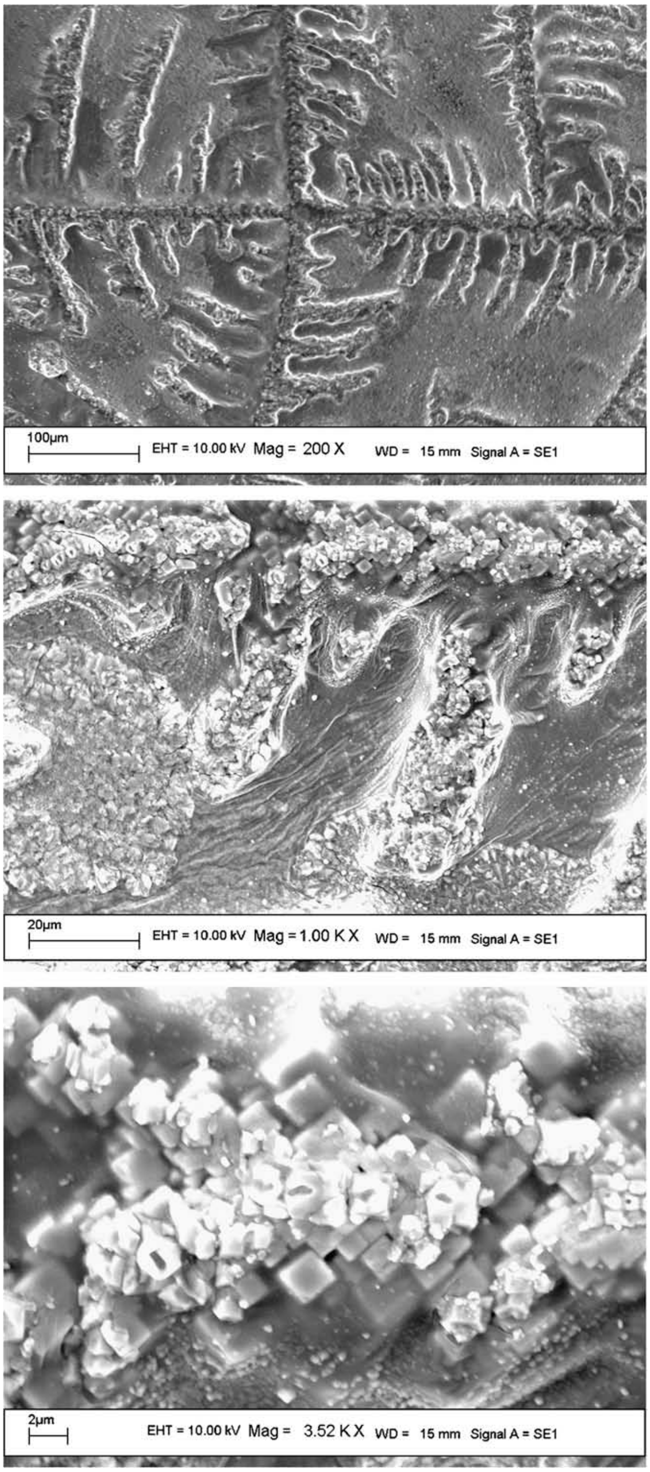

Fig. 6. Scanning electron micrograph (SEM) of dextran showing surface morphology

3.6. Scanning electron microscopic analysis of dextran

Scanning electron micrograph of the dextran showed its surface morphology. The dextran polymer showed a cubical porous or web-like structure (Fig. 6C). Owing to its small pore size distribution, the polymer can hold water and can be used as a texturing agent in food industry. The hydroxyl groups present in the polymer increases the crystallinity of the polymer. The small cubical pore structure may also be responsible for the compactness of the polymer and the stability of the gel structure when subjected to external forces and the maintenance of the texture properties during storage. Polysaccharides are widely used in foods as thickening, gelling, stabilizing, emulsifying, and water-binding agents (Khan, Park, & Kwon, 2007).

4. Conclusions

The results of FT-IR, ^{1}H and ^{13}C NMR and 2D ^{1}H, ^{13}C NMR spectral analysis confirmed that the polysaccharide produced from *L. mesenteroides* NRRL B-640 is a highly linear dextran with $\alpha(1 \rightarrow 6)$ linkages. It is important to screen dextran-producing microorganisms with novel properties with commercial applications. The linear dextrans with $\alpha(1 \rightarrow 6)$ linkage have shown to be one of them. In this study, dextran produced by *L. mesenteroides* NRRL B-640 was purified and its properties were analyzed. It showed a non-Newtonian pseudoplastic behavior. This relatively new biopolymer has unique rheological properties because of its potential of forming very viscous solutions at low concentrations and pseudoplastic nature and can be used as thickening or gelling agent in food. The surface morphology showed that it has cubical pores structure and can be used as thickener in food industry.

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