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Original article

Structural analysis and characterization of a clinically important low molecular weight natural dextran synthesized by *Leuconostoc lactis* KU665298 dextransucrase

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Abstract

Dextransucrases are glucosyltransferases that catalyze the transfer of α-D-glucopyranose residues from sucrose to low molecular weight acceptors, forming oligos and polysaccharides. The main dextransucrase reaction product is dextran, along with the release of fructose. Dextran is a linear polymer of glucose joined mainly by α (1 → 6) links and fewer α (1 → 2), α (1 → 3) and/or α (1 → 4) links. The structural characteristics or the frequency and type of linkage in dextran are dependent on the nature of the enzyme and type of producing microorganism. It has acknowledgeable industrial applications in medical, pharmaceutical, food, textile and chemical industries, depending on its structural characteristics. This paper offers the physical and chemical characterization of a clinically important dextran produced by dextransucrase of a newly isolated strain of *L. lactis* KU665298 in a laboratory fermenter. Structural analysis of dextran by FTIR, ^{1}H NMR, ^{13}C NMR confirmed the presence of a large percentage of α-(1 → 6) linear linkages with very few α-(1 → 3) branch points. The surface morphology and molecular weight distribution of purified dextran has also been included in this study.

Key words: Dextransucrase, dextran, *Leuconostoc lactis*, NMR, FTIR, SEM, gel exclusion chromatography

1. Introduction

The natural substances, which are used in medicines are usually obtained from botanical or microbial sources. Dextran is an example of bacterial homopolysaccharide composed of D-glucose and largely of α-1,6-glucopyranosidic linkages in the main chain (Naessens *et al*., 2005; Vettori *et al*., 2012; Liu *et al*., 2017; Nacher-Vazquez *et al*., 2017). Lactic acid bacteria have a long history of safe use in human consumption and, therefore, among variety of micro-organisms, are most widely explored for the production of microbial exopolysaccharides (Nikolic *et al*., 2012). Fermentation processes for EPS production are quite expensive due to purification costs, giving an advantage to the use of enzymes (Miljkovic *et al*., 2017). Dextran, being an extensively important microbial exopoly-saccharide is synthesized from sucrose by the aid of dextransucrase enzyme. Dextransucrase (sucrose:1, 6-α-D-glucan 6-α-glucosyltransferase, EC 2.4.1.5) is an extracellular protein that belongs to the CAZy family (Carbohydrate Active Enzymes database) of glycoside hydrolases (GH 70) (Kang and Kim, 2013; Leemhuis *et al*., 2013). It is reported to be a large multidomain enzyme of average mass 160 kDa (Monchois *et al*., 1999). Microorganisms belonging to the genera *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Weissella* and *Streptococcus* of Lactobacillaceae and Streptococcaceae families are the main producers of dextransucrase (Di Cagno *et al*., 2006; Majumder *et al*., 2007; Bounaix *et al*., 2010; Hector *et al*., 2015). Dextransucrase catalyzes the synthesis of dextran by transferring D-glucosyl units from sucrose to dextran polymer chain and releasing fructosyl unit free (Jeanes *et al*., 1954; Robyt, 1986; Robyt *et al*., 2008; Li *et al*., 2016). This is called primary or substrate reaction of dextransucrase enzyme.

Dextran has important applications in medical, pharmaceutical, food, textile and chemical industries, depending on its solubility, molecular mass and other structural features (Mehvar, 2000; Naessens *et al*., 2005; Lacaze *et al*., 2007; Sen, 2008; Patel *et al*., 2012). Dextrans of low molecular weight are known as clinical dextran which has applications in pharmaceutical industries while the high molecular weight dextrans can be applied as viscosifying, stabilizing, gelling, sweetening and emulsifying agents in food production (Gan *et al*., 2014). Dextran is the first microbial exopolysaccharide asserted for commercial utility and received approval for sustenance use by the US FDA (Di Cagno *et al*., 2006; Kanimozhi *et al*., 2017). Dextrans have also been used as antithrombogenic agents, treatment for iron deficiency anaemia and drug carriers (Patel *et al*., 2012). Cross linked dextran known as sephadex is widely used as matrix of chromatography columns for purification of proteins in research (Purama and Goyal, 2005). Dextran also plays a role in waste water management by enhancing the flocculation process. In addition to this, silver nanoparticles were also prepared using dextran as both reducing and stabilizing agent (Srinivas and Padma, 2016). Recently, dextran has been used

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in coating of magnetic nanoparticles for their biomedical applications such as a contrast agent in magnetic resonance imaging and gene therapy (Uthaman *et al*., 2015; Tingirikari *et al*., 2017).The broad commercial applications of dextran are due to the versatility in its structural and functional properties.

Two dextran products, *i.e*., Dextran 70 and Dextran 40 are accessible in most countries for clinical purposes (Hashem *et al*., 2014). However, there is a need for the exploration of novel dextran producing microorganisms to meet the requirement of industry. The exploration of new isolates is important because the characteristics of dextran varies from species to species. The high demand and low available amount of dextrans has led to the catalytic synthesis by dextransucrase enzyme.The molecular structure and conformation of dextran are important in determining its bioactivities, which became the focus in recent years (Chen *et al*., 2016; Liu *et al*., 2017). Physical and chemical properties, such as solubility, viscosity, specific rotation, molecular weight and linkages can undergo variations depending on the different dextransucrases produced by different strains of bacteria (Purama, *et al*., 2009; Bai *et al*., 2016; Zhang *et al*., 2016; Guzman *et al*., 2018). Considering the fact that dextran biopolymer has applicability in diverse fields depending upon its structural characteristics; present study was undertaken in which purified dextransucrase (free as well as calcium-alginate immobilized) from a newly isolated strain of *L. lactis* KU665298 has been employed for the production of a low molecular weight dextran in a 3 L laboratory fermenter. Molecular structure, physical properties and conformation of purified dextran were studied by applying various techniques. Clinical applications of *L. lactis* dextran such as anticoagulation and antioxidation properties have also been investigated.

2. Materials and Methods

2.1 Microorganism

A novel dextransucrase producing bacterial strain was isolated from sugarcane baggase sample of sugarcane mill, Karnal, India. It was identified as *L. lactis* KU665298 by 16s rRNA sequencing and nucleotide sequence was submitted to GenBank sequence database.

2.2 Dextransucrase enzyme

Various techniques such as ultrafiltration, precipitation followed by gel permeation chromatography (Sephacryl S-200) were employed for the purification of dextransucrase from *L. lactis* KU665298 (data not shown). The purified dextransucrase enzyme (813 U/mg) from *L. lactis* was immobilized by entrapment method in five different matrices. Calcium-alginate was found to be most suitable matrix for the immobilization of dextransucrase with an immobilization efficiency of 90.7%. It was observed that 2.5% (w/v) of sodium alginate, 0.2 M calcium chloride and 0.09 mg/mL dextransucrase protein were the optimum for the immobilization of dextransucrase in calcium-alginate beads (data not shown).

2.3 Estimation of dextran

The dextran content was determined by phenol-sulphuric acid method (Fox and Robyt, 1991). 2 ml of chilled ethanol was added to 1 ml of sample containing dextran in test tubes and mixed properly for 30 sec. The mixture in test tubes was then centrifuged for 20 min at 15,000 g and the resultant pellet was re-suspended in 1 ml distilled water and to 100 µl of dextran sample 900 µl of distilled water was added to make final volume 1 ml. The mixture was again mixed for 30 sec and 50 µl of 5% phenol was added into it. Subsequently, 2 ml of concentrated H_2SO_4 was added to it and boiled for 10 min and the absorbance was determined at 590 nm using spectrophotometer.

2.4 Production of dextran by purified dextransucrase (free and immobilized) from *L. lactis* in a laboratory fermenter

The dextransucrase of *L. lactis* has been used for production of dextran in a 3 L laboratory fermenter (Bio-spin-031, India) under the optimized conditions. Free dextransucrase (813 U/mg) was incubated with 1 L of 8% (w/v) sucrose prepared in sodium acetate buffer (25 mM, pH 5.5, containing 0.1 mM $CaCl_2$) at 30ºC for 24 h. The same amount of the dextransucrase was immobilized by entrapment in calcium alginate matrix. Dextran was produced by incubating immobilized dextransucrase with 12% (w/v) sucrose prepared in sodium acetate buffer (25 mM, pH 5.5, containing 0.1 mM $CaCl_2$) at 35ºC for 24 h. The samples were withdrawn from fermenter at regular interval of 1 h to determine the amount of dextran synthesized. The dextran prepared was then precipitated by ethanol (75%, v/v) and dextransucrase protein was removed by precipitation with TCA (25%, w/v).

2.5 Purification of dextran synthesized in labaratory scale fermenter

The synthesized dextran was precipitated using 75% (w/v) ethanol and re-suspended in water, after the completion of incubation. It was treated with 5 ml TCA (25%, w/v) to remove protein content. Ethanol precipitation was repeated twice to obtain highly purified dextran. The polysaccharide content was determined by phenol-sulphuric acid (Fox and Robyt, 1991). The precipitate was dissolved in the warm water and vacuum dried by lyophilization. The dextran concentration was noted once the product dried completely. The amount of dextran produced was measured on dry weight basis as well as spectrophotometrically.

2.6 Characterization of dextran produced by *L. lactis* dextransucrase

2.6.1 Solubility of *L. lactis* dextran

Solubility of a dextran of *L. lactis* was checked in water and various organic solvents (methanol, ethanol, glycerol and polyethylene glycol).

2.6.2 Viscosity of *L. lactis* dextran

Viscosity of dextran of various concentrations (0.1, 0.2, 0.3, 0.4, 0.5%, w/v) was determined by jacketed Ostwald Viscometer.

2.6.3 Molecular weight determination of *L. lactis* dextran

The average molecular weight of dextran produced from biotransformation of sucrose by purified dextransucrase from *L. lactis* was determined by gel permeation chromatography using blue dextran 2000 as standard. The molecular mass determination of unknown dextran was made by comparing the ratio of V_e/V_o for the dextran in question to the V_e/V_o of dextran standards of known molecular mass (V_e and V_o serve as elution and void volume, respectively). The V_o of a given column is based on the volume of effluent required for the elution of a blue dextran (molecular mass of about 2000 kDa). However, the V_e for dextrans was established

by the volume measurement of effluent collected from the point of dextran application to the centre of the effluent peak. Plotting the molecular weights of the known molecular masses of dextran standards versus their respective V_e/V_o values produces a linear calibration curve. The dextran standards (1 ml) were loaded on glass column (50×1.5 cm) packed with Sephacryl-S-2000 HR (40 ml) having 2500 cut off. It was eluted with 0.01 M phosphate buffer (pH 7.0) at a constant flow rate of 30 ml/h. The fractions (5 ml was collected and the elution of the standard was followed by measuring its absorbance at 590 nm. All standard dextran fractions were eluted in void volume (V_o). Further, the column was washed properly with 150 ml of potassium phosphate buffer (pH 7.0, 10 mM). The sample (1 ml) containing 2.0 mg purified dextran was loaded to the column. The same flow rate (0.5 ml/min) was used as it was for the blue dextran and the dextrans of known molecular weights (standards) and fractions of 5 ml were collected. The V_e of *L. lactis* produced dextran was determined and V_e/V_o for the unknown dextran was calculated and its molecular mass was determined from the standard curve.

2.6.4 Fourier transform infrared spectrometric (FT-IR) analysis of *L. lactis* dextran

FTIR is the most powerful tool for identifying types of chemical bonds. The wavelength of light absorbed is characteristic of the chemical bond which could be seen in this annotated spectrum. The chemical bonds in a molecule can be determined by interpreting this infrared absorption spectrum. The FT-IR spectrum using spectrometer (Perkin-Elmer Spectrum RX-IFT-IR Spectrum) was also recorded for purified dextran obtained from dextransucrase of *L. lactis* and standard dextran in a KBr pellet.

2.6.5 Nuclear magnetic spectrum (NMR) analysis of *L. lactis* dextran

NMR was performed in a multinuclear FT NMR model Avance-II 400 NMR (Bruker) spectrometer. The purified dextran obtained from *L. lactis* was vacuum dried and exchanged with deuterium by successive lyophilization steps in D_2O (99.6% atom 2 h) and then dissolved in 0.5 ml of D_2O (10 mg/ml). Tetramethylsilane (TMS) was used as an integral reference. 1D ^1H NMR and ^{13}C NMR spectra of dextran were recorded at a base frequency of 100 MHz for analysing in the linkage composition. NMR spectrum of standard dextran was also recorded.

2.6.7 Scanning electron microscope analysis of *L. lactis* dextran

A sample of the dried polymer dextran produced by *L. lactis* dextransucrase was attached to the SEM stub with double-sided tape. The surface of the sample was viewed in SEM (operated at JSM-6100) 5.0Kv.

2.7 Clinical applications of dextran produced by *L. lactis* dextransucrase

2.7.1 Dextran as an anticoagulating agent

The modified dextran (sulphate ester) has anticoagulant properties similar to those of heparin. Anticoagulation activity of native dextran was also studied by preparing 0.25% (w/v) of dextran as stock solution in distilled water. 1 ml of 0.25% (w/v) stock solution was taken in the test tube and 1 ml of human blood was added. Further, 1 ml of distilled water in place of dextran were taken in another test tube which act as control. The test and control tubes were shaken well and kept at 37°C for 1 h and effect of dextran on the blood coagulation was observed.

2.7.2 Dextran as an antioxidizing agent

The antioxidants react with the stable free radical 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (deep violet color) and convert it to 1, 1-diphenyl-2-picryl hydrazine with decolouration (Fahim *et al*., 2017). The bleaching of a purple colored methanol solution of DPPH was the measure for the hydrogen atom or electron donation ability of *L. lactis* dextran. Purified dextran at various concentrations ranging from (50-450 µg/ml) was mixed with 1 ml of freshly prepared 0.5 mM DPPH methanolic solution and 2 ml of distilled water. The mixture was shaken vigorously and incubated in dark at 37°C for 30 min and decrease in A_{517} values was recorded. Ascorbic acid, a stable antioxidant was used as a synthetic reference. The radical scavenging activities of samples expressed as percentage inhibition of DPPH were calculated according to the formula.

$$\text{Inhibition percentage} = \frac{[AB - AA] \times 100}{AA}$$

where, AB and AA are the absorbance values of the blank sample and of the test samples checked after 30 min, respectively.

3. Results and Discussion

3.1 Production of dextran by purified dextransucrase (free and immobilized) from *L. lactis* in a laboratory fermenter

Dextran is an extracellular bacterial homopolysaccharide (Sidebotham, 1974; Monsan *et al*., 2001), the main chain of which is composed of contiguous (1, 6)-linked d-glucopyranose residues and branches, stemming mainly side-chains of α-(1,6) glucose units attached by α-(1,3) branch linkages to α-(1,6)- linked chains (Seymour and Knapp, 1980; Robyt, 1995; Dols *et al*., 1998; Buchholz and Monsan, 2001; Naessens, *et al*., 2005). Some dextrans have α-(1, 2) or α-(1,4) branch linkages, depending on the bacterial source. The exact structure of each type of dextran depends on the degree of branching, involving α-(1, 2), α-(1,3) and α-(1, 4) linkages (Seymour and Knapp, 1980), which depends on the specific microbial strain and, hence, on the specificity of dextransucrase involved (Jeanes *et al*., 1954; Purama *et al*., 2009; Bai *et al*., 2016; Liu *et al*., 2017). In order to gain a better understanding of physicochemical, structural, and rheological parameters of the dextran, the present study focused on the production of dextran by employing *L. lactis* dextransucrase and characterization of purified dextran.

The purified dextran was vacuum dried using lyophilizer and the amount of dextran was determined spectrophotometrically as well as on dry weight basis (Figure 1). The dextran production on dry weight basis was found to be 5.10 mg/ml (5.36 mg/ml spectrophotometrically) by free dextransucrase from *L. lactis* after 24 h of incubation. Whereas, the calcium-alginate immobilized dextransucrase from *L. lactis* produced 2.02 mg/ml dextran on dry weight basis at 24 h of reaction. However, 2.20 mg/ ml dextran was found to be synthesized when estimated spectrophotometrically.

Figure 1: Lyophilized dextran produced by dextransucrase of *L. lactis*.

3.2 Characterization of purified dextran synthesized by *L. lactis* dextransucrase

3.2.1 Solubility of *L. lactis* dextran

Dextran produced by dextransucrase of *L. lactis* was white powder. Solubility of dextran was studied in the water and different organic solvents (PEG, glycerol, ethanol, methanol, *etc*). Dextran was rapidly soluble in PEG, glycerol and water. However, it was insoluble or gets precipitated in other organic solvents (alcohols and methanol). Similar results have been reported for dextran synthesized by *L. mesenteroides* NRRL B-512(F) dextransucrase (Senti *et al*., 1955; Bovey, 1959). However, it has also been reported that water may not be a good solvent for dextran due to the chemical structure of the polymer and the dense hydrogen bonding of water (Ali, 2004). Low solubility of *L. mesenteroides* NRRL B-1149 dextran in water was associated with the presence of high percentage of α-(1-3) linkages (Shukla *et al*., 2011).

3.2.2 Viscosity of *L. lactis* dextran

The viscosity of dextran produced from dextransucrase of *L. lactis* was found to be 1.06, 0.94, 0.89, 0.83, 0.79 cp (centipoise) at 0.5, 0.4, 0.3, 0.2, 0.1 (%, w/v) concentration of dextran, respectively (Figure 2). This confirmed that dextran has gel forming nature and with the increase in concentration, it forms viscous solution in water. Hence, dextran can be applied as viscosifying, stabilizing, gelling, and emulsifying agent in food production (Lacaze *et al*., 2007; Patel *et al*., 2011). Cross linked dextran known as sephadex is widely used for purification of proteins in research (Purama and Goyal, 2005).

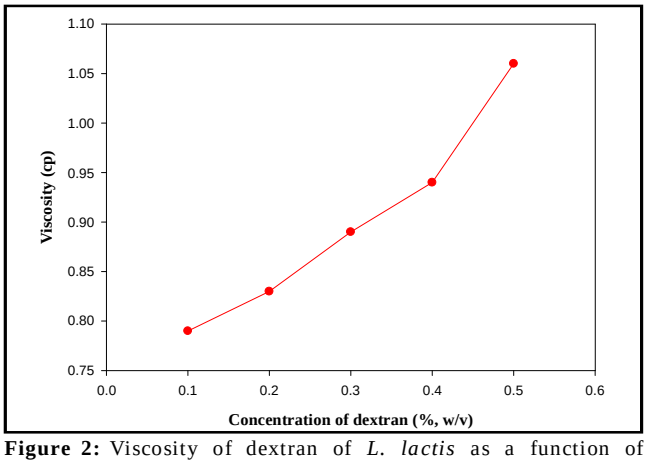

Figure 2: Viscosity of dextran of *L. lactis* as a function of concentration.

3.2.3 Molecular weight determination of *L. lactis* dextran

Gel exclusion chromatography was performed using Sepharcryl-S-200 HR to determine the average molecular weight of dextran produced from dextransucrase of *L. lactis*. Elution pattern of blue dextran indicated that the void volume of column was approximately 60 ml (Figure 3). The molecular mass determination of unknown dextran was made by comparing the ratio of V_e/V_o for the dextran in question to the V_e/V_o of dextran standards of known molecular mass (V_e is the elution volume and V_o is the void volume), depicted in Figure 4. Plotting the molecular weights of the known molecular masses of dextran standards versus their respective V_e/V_o values produces a linear calibration curve. The V_e/V_o of *L. lactis* dextran was calculated and its molecular mass was determined from the standard curve. The molecular mass of the dextran produced by the action of *L. lactis* dextransucrase was found to be around 55 kDa (Figure 5). It has been reported that dextrans with relatively low molecular weight (20, 40, and 70 kDa) are excellent plasma extenders (Spence *et al*., 1952; Bark and Grande, 2014). The low molecular weight dextrans have been used widely due to their reduced risk of side-effects such as hemorrhages or allergic responses. They have also been utilized as anti-thrombotic agents to prevent deep vein thrombosis (Schwarz *et al*., 1981; Klotz and Kroemer, 1987). In addition, dextrans have been demonstrated in organ perfusion experiments to influence the binding of leucocytes to microvascular endothelium *in vivo* (Qader and Aman, 2012; Hashem *et al*., 2016).

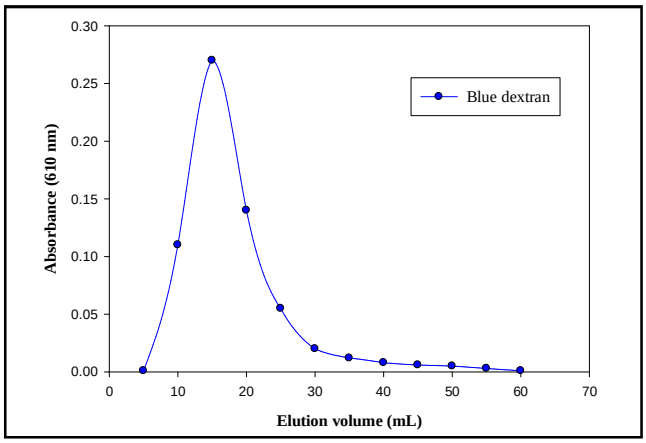

Figure 3: Molecular mass distribution of blue dextran used as reference.

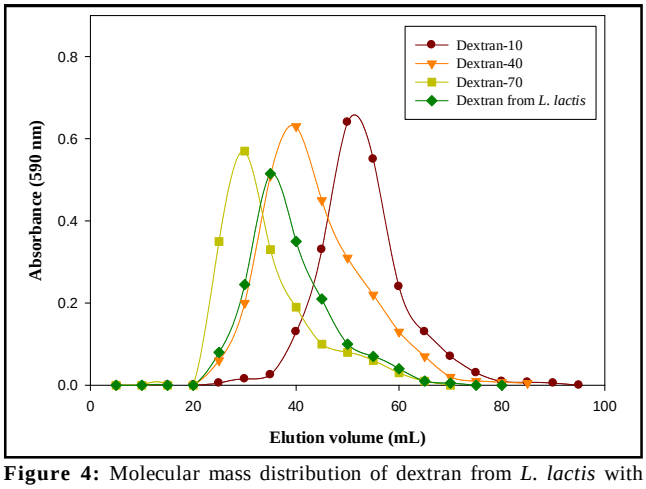

Figure 4: Molecular mass distribution of dextran from *L. lactis* with reference to dextran-10, Dextran-40, and Dextran-70.

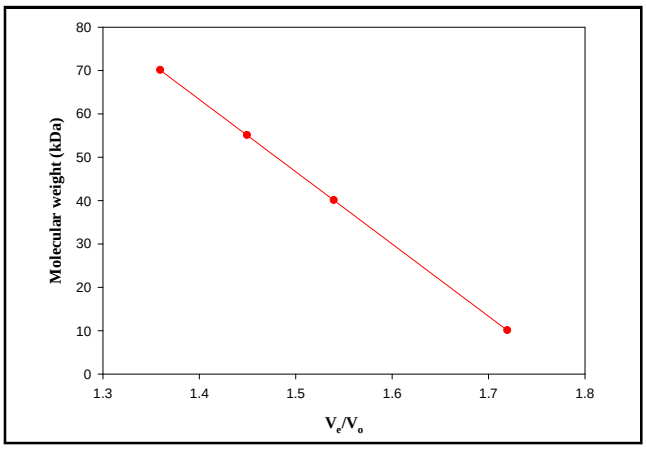

Figure 5: Molecular weight vs V_e/V_o.

3.2.4 Fourier-transform infrared spectroscopic (FTIR) analysis

The FT-IR spectra were used to characterize the types of linkages and functional groups of dextran synthesized by dextransucrase obtained from *L. lactis* KU665298. Spectroscopic analysis by FT-IR also establishes the purity of dextran synthesized. The FTIR spectra of standard dextran and purified dextran of *L. lactis* were found to be related (Figures 6 a and b). Figure 6(b) shows the peaks at 3346 cm^{-1} for -OH, at 2929 for $-CH_2$, at 1647 cm^{-1} for -COO group. These results are supported by many other reports in which, FTIR spectrum of the EPS indicated that the polysaccharide contained a significant number of hydroxyl groups as it displayed a broad and intense stretching peak around 3400 cm^{-1} (Wang *et al*., 2010; Gan *et al*., 2014; Mukhopadhyay *et al*., 2014).The stretching band around 2929 cm^{-1} was due to C-H stretching vibration (Melo *et al*., 2002; Cao *et al*., 2006; Liu *et al*., 2007).The absorption peak at 919 cm^{-1} indicates the presence of α-glycosidic bond. The bands found at 1153, 1104 and 1017 cm^{-1} are due to valent vibrations of C-O and C-C bonds and vibrations of CCH, COH and HCO bonds. The bands at 1153 cm^{-1} is assigned to valent vibrations of C-O-C bonds and glycosidic bridge. Further, the peak at 1017 cm^{-1} is due to the great chain flexibility present in dextran around the α-(1,6) linkages (Shingel, 2002). Therefore, FTIR spectral analysis of *L. lactis* dextran showed that it contains α-(1,6) glycosidic linkages. This was further confirmed by ^{1}H NMR and ^{13}C NMR spectral analysis.

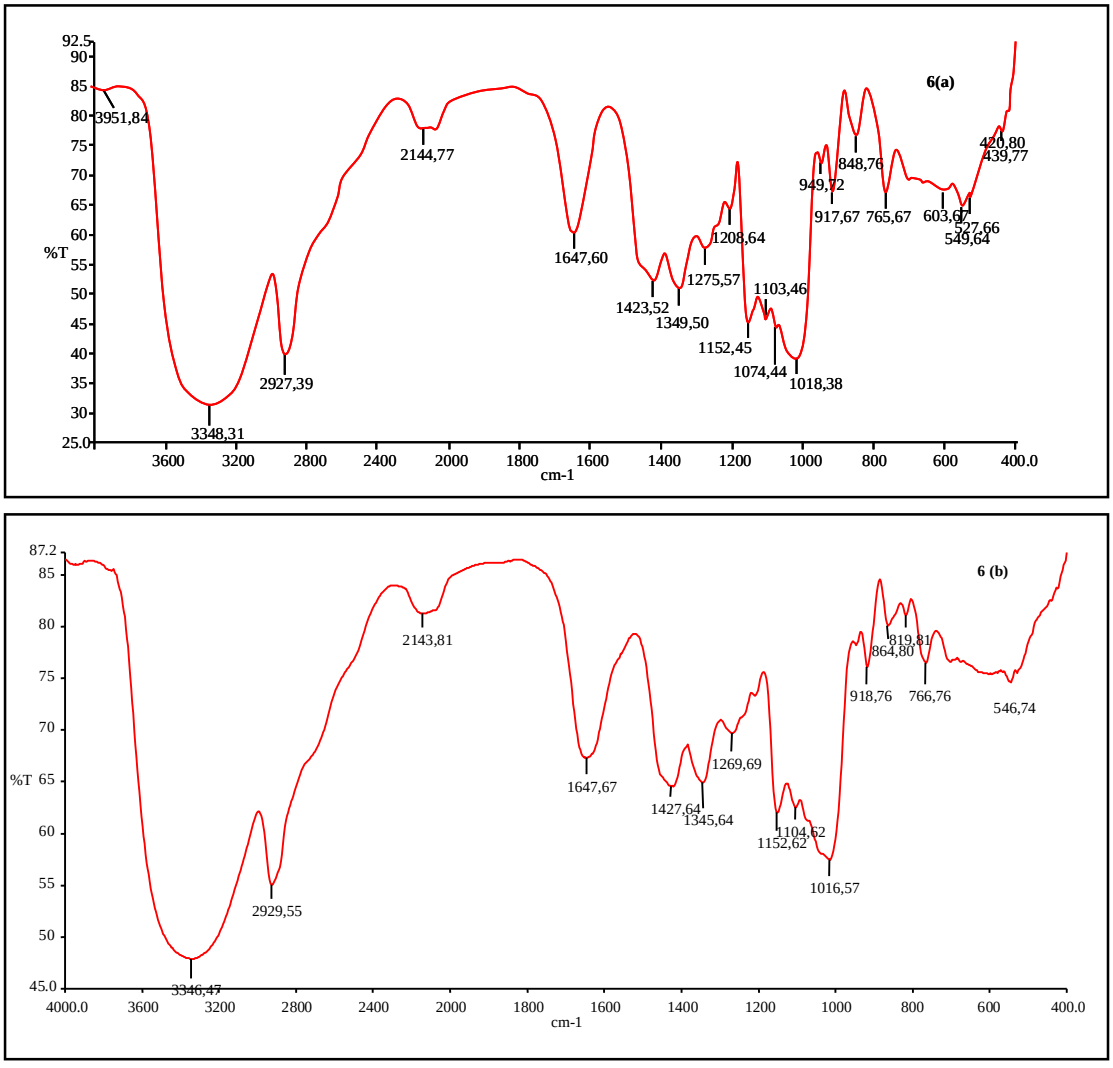

Figure 6: FT-IR (KBr) spectrum of (a) standard dextran-10 and (b) dextran synthesized by dextransucrase from *L. lactis*.

3.2.5 ^{1}H NMR

The anomeric proton resonance for the 400 MHz ^{1}H NMR spectra of dextran-10 and *L. lactis* dextran have been given in (Figures 7 a and b). ^{1}H spectra of the *L. lactis* dextran showed resonance between 3.43 and 4.92, which confirmed the presence of anomeric carbon. The peak at 4.92 ppm is due to the H-1 of the (1-6) glucosyl residues of main chain (Bounaix *et al*., 2009; Kanimozhi *et al*., 2017).The two signals that appear centered at 5.25 and 5.26 ppm represent important structural characteristics of α-(1,3)-linked-d-glucosyl residues (Maina *et al*., 2008; Bounaix *et al*., 2009) (Figure 7 b). The integration of signals 5.25 and 4.92 gives the percentage of α-(1,3)-linked d-glucosyl and α-(1,6)-linked d-glucosyl linkages, respectively. ^{1}H NMR spectra confirmed the presence of a large percentage of α- (1-6) linear linkages with very few α-(1-3) branch points in the structure of *L. lactis* produced dextran. In a previous study, the α-1,6-glycosidic linkage in the main chains was demonstrated at a peak of 4.93 ppm, whereas α-1, 3-glycosidic link-age in the branch is located at approximately 5.20 ppm (Gan *et al*., 2014). Similar results have been reported by other authors (Seymour *et al*., 1979; Vettori *et al*., 2012; Dong *et al*., 2013).

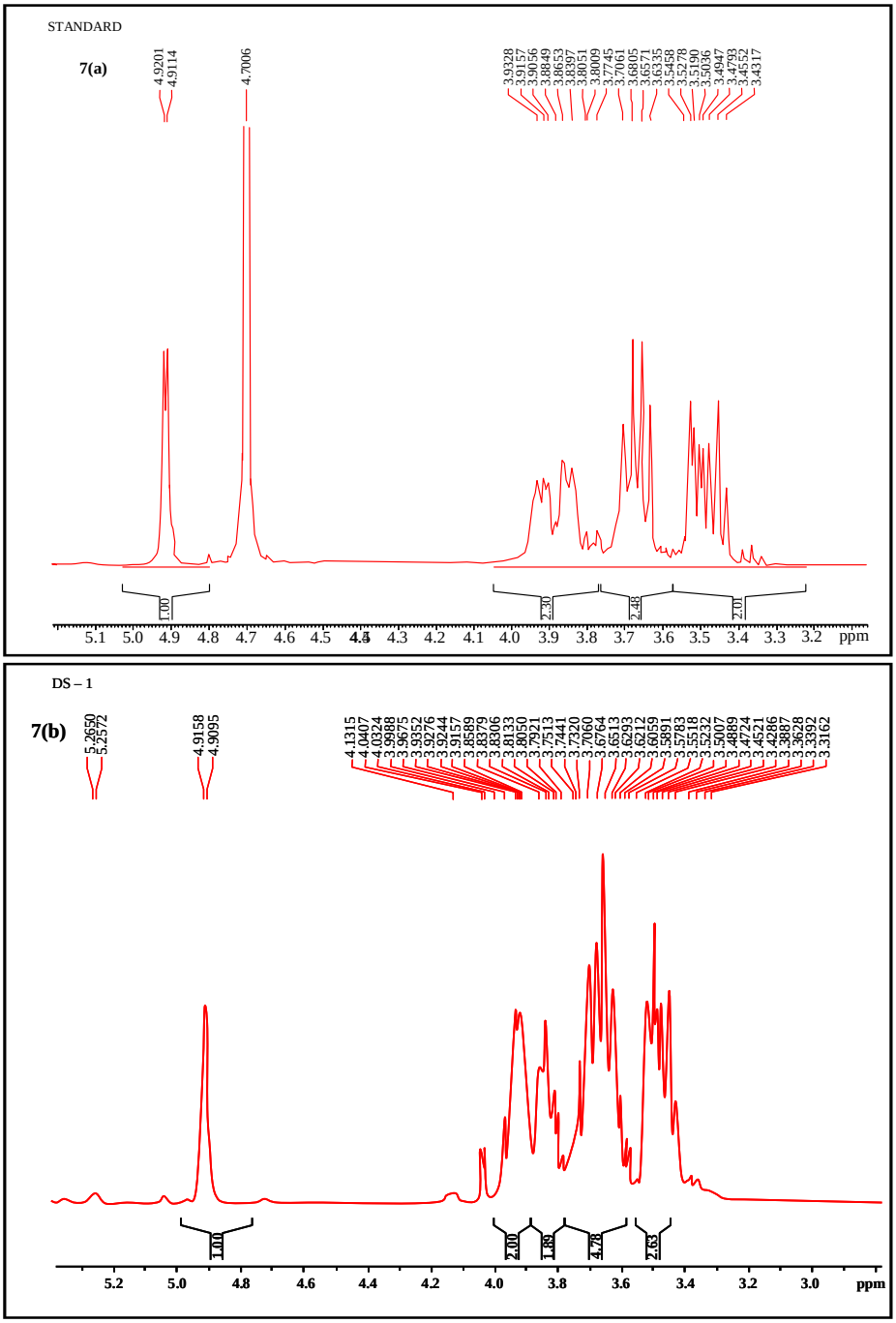

Figure 7: ^{1}H NMR (400 MHz D_2O) spectrum of (a) dextran-10 and (b) dextran produced from the purified dextransucrase of *L. lactis*.

3.2.6 ^{13}C NMR

The dextran showed six prominent ^{13}C NMR resonance at 100 MHz 98.05, 97.70, 73.39, 71.39, 69.18 and 65.53 ppm (Figures 8 a and b), which was a characteristic of linear dextran. The major resonance in the anomeric region occurs at 97.70 ppm showed that the C-1 is linked (Vettori *et al*., 2012). Another intense signal at 62.65 confirmed that most of C-6 were also linked, and this proved that glucose residues in dextran were linked by α-(1,6) glycosidic bond. There were additional peaks at 75 to 85 ppm regions, which indicate that the dextran synthesized by the purified dextransucrase from *L. lactis* has branching structure (Du *et al*., 2017). The spectra obtained from both, FTIR and NMR spectroscopy confirmed that purified dextran from *L.lactis* contains α-(1, 6) glycosidic linkages in the main chain with α-(1, 3) branch point in its structure.

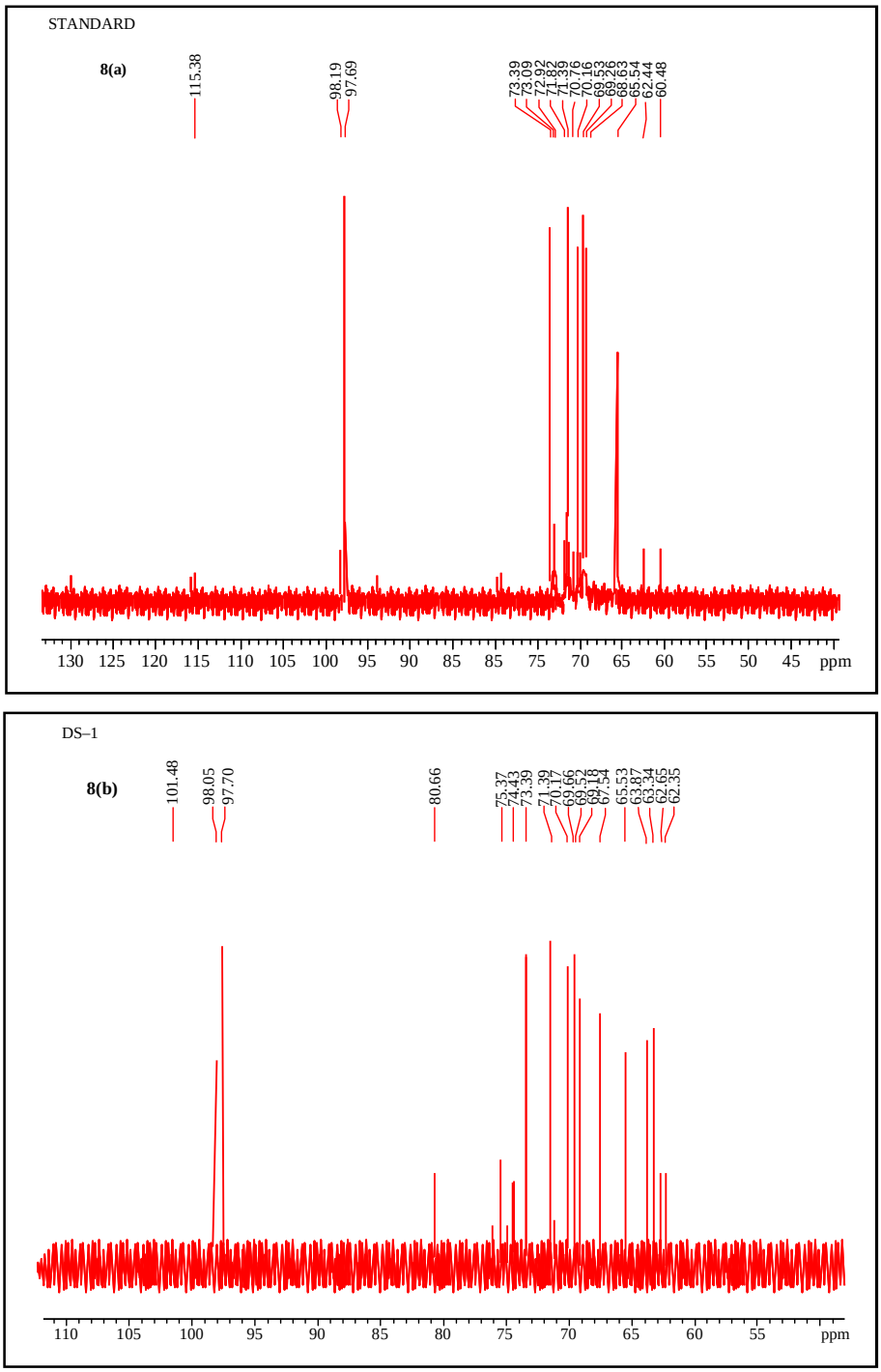

Figure 8: (a) ^{13}C NMR (1MHz, D_2O spectrum of dextran-10 and (b) dextran produced from the produced from the purified dextransucrase of *L. lactis*.

3.2.7 SEM analysis of dextran of *L. lactis*

Surface morphology of the dextran was analysed by SEM (Figure 9). At different resolutions, a highly compact mesh of dextran sample was observed with small pore size distribution, predicting that the dextran can seize substantial amount of water molecules in order to facilitate an even texture when solubilized in water based solutions. Water soluble dextran which gives a smooth creamy texture is most likely utilized in food industries as texturing, thickening, gelling, stabilizing and emulsifying agents (Khan *et al*., 2007; Siddiqui *et al*., 2014).

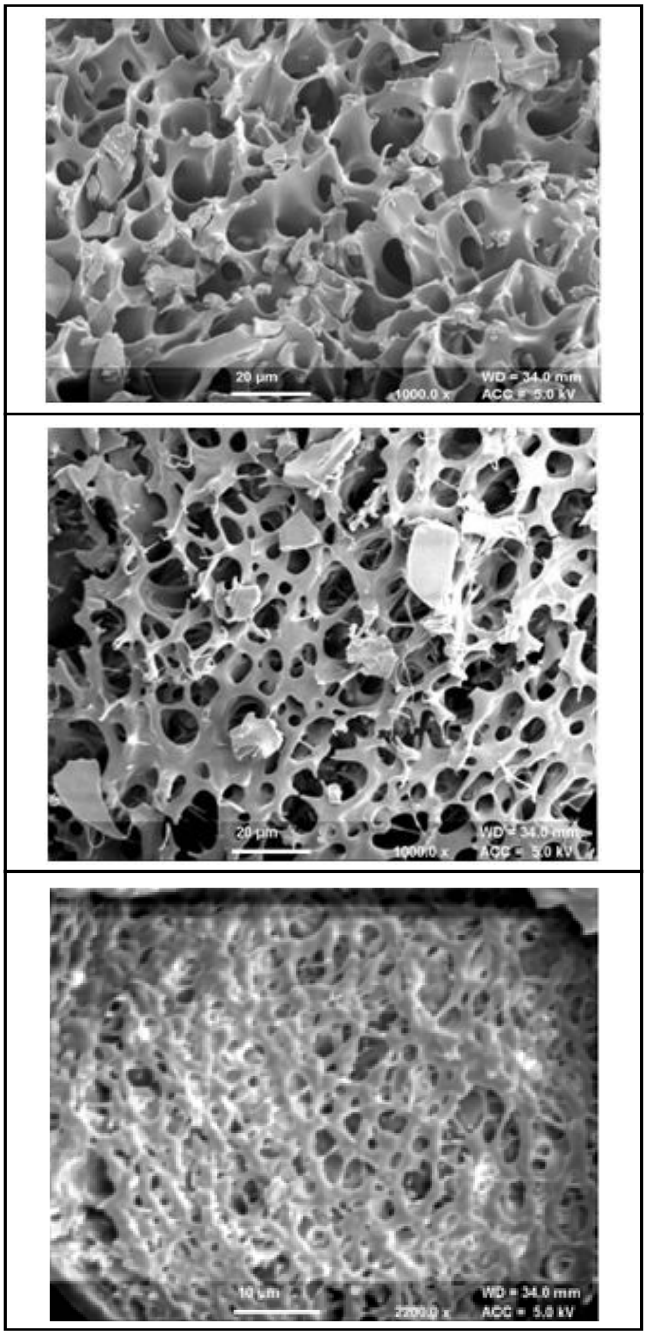

Figure 9: Scanning electron microscope of dextran produced from *L. lactis* showing its surface morphology.

3.3 Clinical applications of low molecular weight dextran synthesized by *L. lactis* dextransucrase

3.3.1 Dextran as an anticoagulating agent

Anticoagulant activity with low toxicity has been demonstrated using the dextran derivative, dextran sulfate (Robyt, 1986; Di Cagno *et al*., 2006; Bashari *et al*., 2013). Native dextran from *L. lactis* may not be an efficient anticoagulating agent as modified dextran (sulphate ester), but it was found to slow down the process of agglutination. *L. lactis* produced dextran was found to prevent the clot formation in the test sample of blood when observed for 1 h (Figure 10).

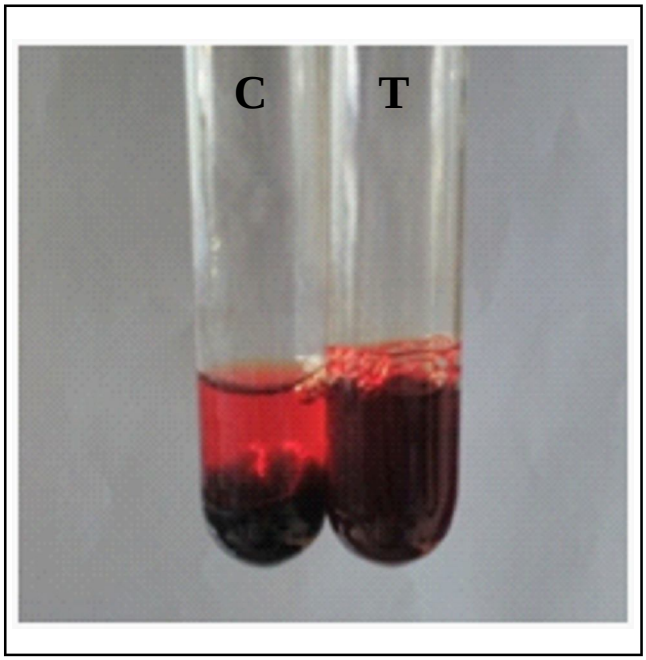

Figure 10: *L. lactis* dextran delaying clot formation in test (T) and in control (C) clot formation in absence of dextran.

3.3.2 Dextran as an antioxidizing agent

Dextrans whose monosaccharides present exclusively in the α configuration have interesting antioxidant property (Gao *et al*., 2011). In a recent study, a low molecular weight dextran (dextran 40) has proved as an efficient antioxidant (Vinicius *et al*., 2016). The DPPH free radical scavenging assay is a well-established method for determination of antioxidant activity of any molecule. The method is based upon the reduction of methanol DPPH solution in the presence of hydrogen donating antioxidant due to formation of the non-radical form DPPH-H by the reaction (Joshi *et al*., 2017; Pant *et al*., 2017). The varying concentrations (50-450 µg/ml) of purified dextran in distilled water were used for the evaluation of antioxidant activity. The low molecular weight dextran from *L. lactis* was proved to be a mild antioxidant. Figure 11 shows the total antioxidant scavenging potential of DPPH where ascorbic acid is used as the positive scavenger and measured at various concentrations. Maximum antioxidant activity (32%) was found with 300 µg/ml dextran of *L. lactis*. However, with subsequent increase in dextran concentration, its antioxidant activity remained constant.

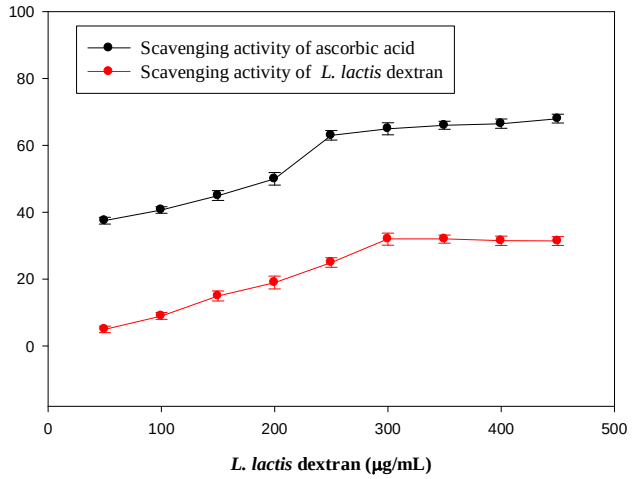

Figure 11: Antioxidant activity of *L. lactis* produced dextran by DPPH method.

4. Conclusion

Dextrans are available in multiple molecular weights but the low molecular weight dextrans have many therapeutic applications. It acts as plasma expander and lowers viscosity of blood and improves flow. In part, the improvement in flow is the result of hemodilution and also decreases the serum fibrinogen and clotting factors. The potential of *L. lactis* KU665298 dextransucrase for the production of dextran was investigated in this study. A low molecular weight dextran of approximately 55 kDa was synthesized by the action of *L. lactis* dextransucrase. This justified the clinical potential of *L. lactis* dextran, which was also demonstrated by its anticoagulating and anti-oxidizing properties. In addition to this, porous structure and water solublenature of dextran also suggest its competence in food industries.

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Conflict of interest

We declare that we have no conflict of interest.

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