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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.

Table 1
Characterization of dextran: (A) effect of various fermentation parameters on the production of dextran from *L. mesenteroides* KIBGE-IB22 (wild type) and *L. mesenteroides* KIBGE-IB22M20 (mutant); (B) physicochemical properties of dextran.

	L. mesenteroides KIBGE-IB22 (wild type)		*L. mesenteroides* KIBGE-IB22M20 (mutant)	
	Dextran (g)	% Conversion[e]	Dextran (g)	% Conversion[e]
(A) Fermentation parameters				
Time course[a] (h)				
6	0.54	5.40	3.06	30.6
12	1.53	15.3	4.37	43.7
18	1.90	19.0	4.37	43.7
24	2.41	24.1	4.37	43.7
30	2.41	24.1	4.37	43.7
36	2.41	24.1	4.37	43.7
Substrate[b] (%)				
2.0	0.40	20.0	0.74	37.0
5.0	1.11	22.2	2.10	42.0
10.0	2.41	24.1	4.37	43.7
15.0	3.52	23.5	7.09	47.1
20.0	4.46	22.3	9.00	45.0
25.0	4.95	19.8	10.45	41.8
30.0	5.10	17.0	10.32	34.4
Temperature[c] (°C)				
10	0	0	0	0
15	2.06	20.5	0.34	2.30
20	2.25	22.2	4.97	33.0
25	2.41	24.1	7.09	47.3
30	2.09	20.9	6.25	41.6
35	0.53	5.30	2.51	16.7
40	0	0	0.32	2.13
pH[d] (before autoclaving)				
5.0	0.12	1.20	0.65	4.35
6.0	0.96	9.60	6.30	42.0
7.0	2.10	21.0	7.07	47.1
7.5	2.41	24.1	7.09	47.3
8.0	2.25	22.5	6.76	45.0
9.0	2.06	20.6	6.74	44.9
10.0	0	0	0	0
(B) Physicochemical parameters				
Total protein[f] (%)	3.80	2.20		
Reducing sugar[f] (%)	0.36	0.12		
Total carbohydrate[g] (%)	79.0	83.0		
Viscosity[h] (cp)	342	286		
Ash content (%)	11.20	9.03		
Moisture content (%)	–	–		
pH[f]	6.60	6.90		

[a] Dextran produced using 10.0 g dL^{-1} sucrose in a fermentation medium with pH 7.5 at 25 °C for both wild and mutant.
[b] Dextran produced using a fermentation medium with pH 7.5 at 25 °C for 24 and 12 h for wild and mutant, respectively.
[c] Dextran produced using 10.0 g dL^{-1} and 15.0 g dL^{-1} sucrose in a fermentation medium with pH 7.5 for 24 and 12 h for wild and mutant, respectively.
[d] Dextran produced using 10.0 g dL^{-1} and 15.0 g dL^{-1} sucrose in a fermentation medium at 25 °C for 24 and 12 h for wild and mutant, respectively.
[e] Calculation is based on the conversion of sucrose into dextran.
[f] 1.0% dextran solution.
[g] 0.2% dextran solution.
[h] 5.0% dextran solution at 24 °C.

Current investigation deals with the production and structural characterization of a high molecular weight dextran produced by a natural isolate, *L. mesenteroides* KIBGE-IB22 and its mutant *L. mesenteroides* KIBGE-IB22M20. Isolation, identification and screening of both strains on the basis dextran production and over-expression of dextransucrase after mutation were reported previously (Siddiqui et al., 2013). Several other generated mutants were also screened for dextran production but selected mutant (KIBGE-IB22M20) produced dextran approximately 1.18 times higher as compared to all other mutants and the wild type. The selected mutant showed its stability and was consistent in the overproduction of dextran after several repeated sub-culturing (data not shown). It has been reported earlier that UV irradiation increased dextransucrase production which ultimately leads to increased dextran production (Kim & Robyt, 1995; Kothari, Tyagi, Patel, & Goyal, 2011; Patel et al., 2011). Therefore, production of dextran from both these strains was optimized and then characterized on the basis of structural and physicochemical properties.

The effect of different operating conditions as well as substrate concentration on dextran production by several other variants of *L. mesenteroides* have been well documented (Qader & Aman, 2012; Sarwat et al., 2008; Aman et al., 2012; Vedyashkina, Revin, & Gogotov, 2005). Therefore, the production of dextran by KIBGE-IB22 and KIBGE-IB22M20 were also optimized by varying different fermentation parameters for the maximum production of dextran (Table 1A). Wild strain showed maximum conversion of sucrose into dextran (24.1%) after 24 h while mutant strain showed more conversion of sucrose (43.7%) into dextran in less than 12 h as compared to the wild type. The reason for this increase in the dextran yield by mutant in lesser time period was further clarified after studies were carried out on structural analysis of dextran produced by wild and mutant strains. It was found that KIBGE-IB22M20 consumed more substrate for dextran production (15.0 g dL^{-1}) as compared to KIBGE-IB22, whereas other parameters including fermentation temperature and pH stayed same for both the strains for the production of dextran. Data presented in Table 1B shows

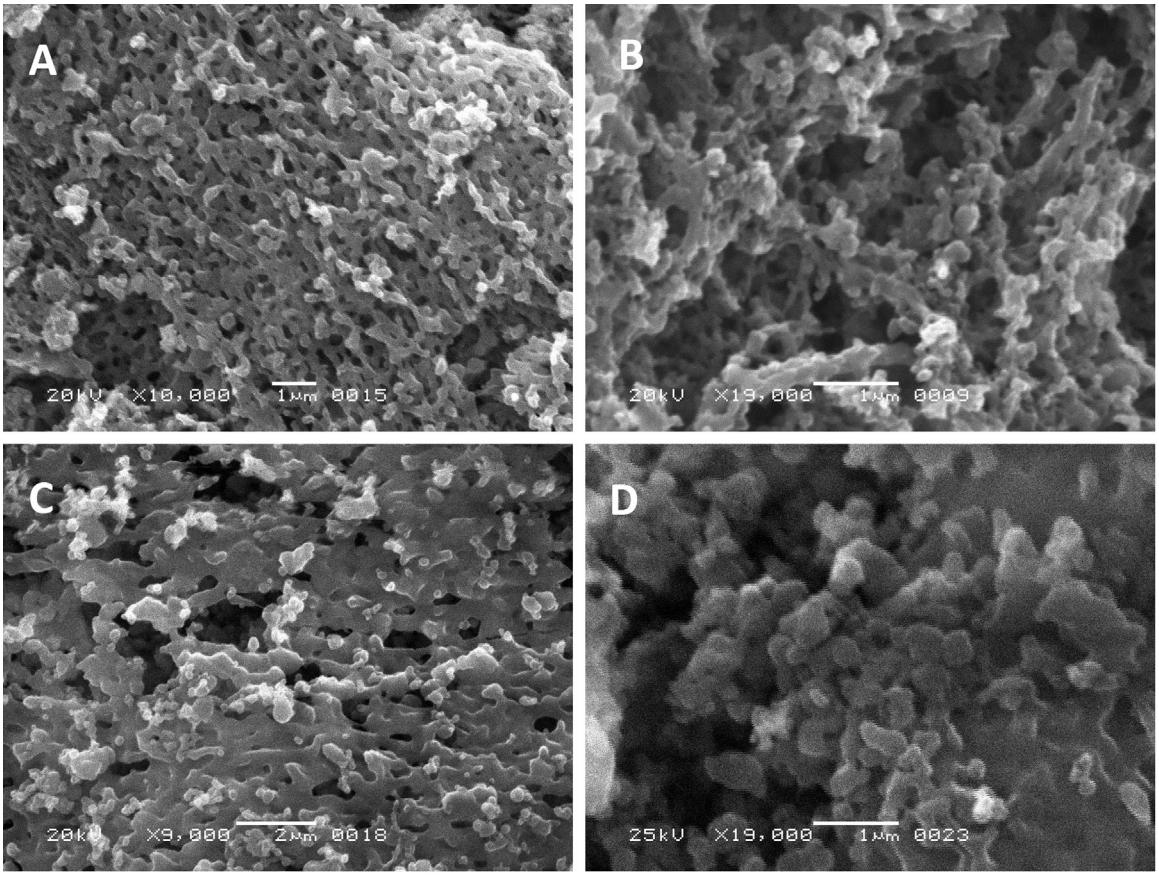

Fig. 1. Scanning electron microscopy of dextran produced from *Leuconostoc mesenteroides*. (A and B) SEM of dextran produced by KIBGE-IB22; (C and D) SEM of dextran produced by KIBGE-IB22M20.

the comparison of the physico chemical parameters of dextran produced by both strains.

3.1. Surface morphology of dextran

Morphological structure of dextran produced by both strains was analyzed using SEM. Surface morphology of the dextran from KIBGE-IB22 and KIBGE-IB22M20 is presented in Fig. 1. At different resolutions, a highly compact mesh was observed in both cases with small pore size distribution predicting that both 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, Park, & Kwon, 2007).

3.2. Molecular mass of dextran

Size exclusion chromatography was performed for the determination of average molecular mass of dextran produced by wild and mutant using blue dextran and industrial grade dextran as standards. Average molecular mass calculated for the dextran produced by KIBGE-IB22 and KIBGE-IB22M20 was in the range between 15,000,000–20,000,000 Da and 25,000,000–40,000,000 Da, respectively (Fig. 2). It was found that the average molecular weight of dextran from mutant was not only higher than the wild type but was also higher than the industrial grade dextran.

3.3. Characterization of dextran

Structural characterization of dextran produced by KIBGE-IB22 and KIBGE-IB22M20 was performed using FTIR and NMR which are also widely used to study the full structural features of various EPS.

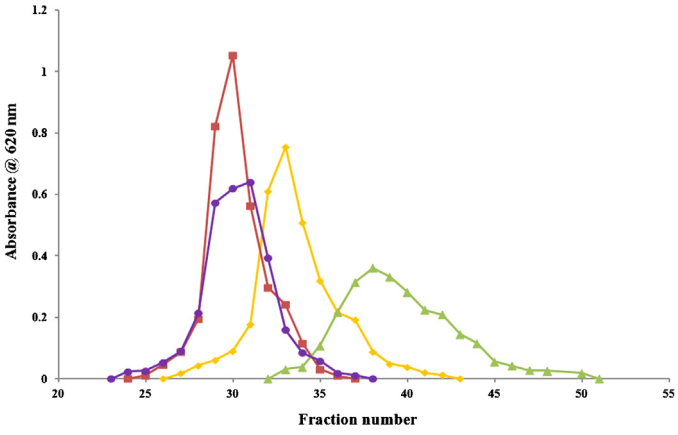

Fig. 2. Determination of average molecular weight of dextran produced by wild and mutant strains of *Leuconostoc mesenteroides*. All the samples of dextran were eluted using the same conditions. 2 mg/mL of each sample used for size exclusion chromatography. Blue dextran (▲), industrial dextran (◆), dextran from KIBGE-IB22M20 (■, dextran from KIBGE-IB22 (●).

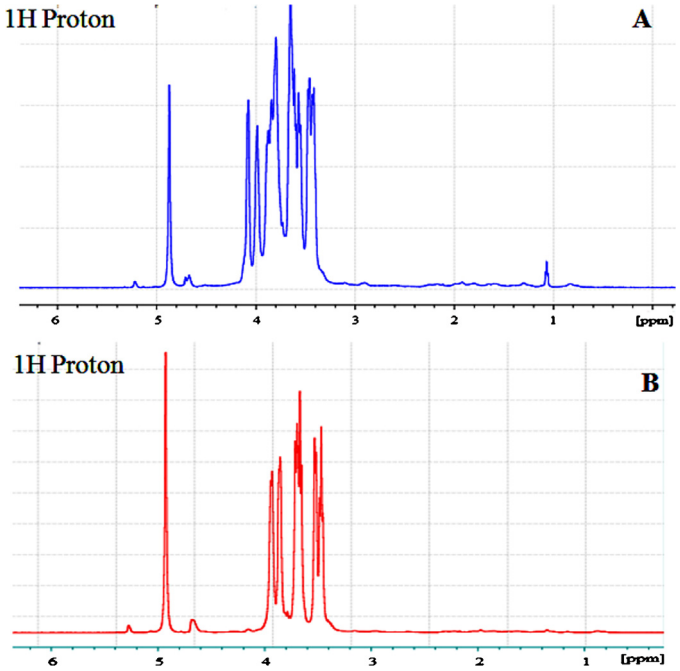

Fig. 3. ^{1}H NMR spectra of polysaccharides extracted from *Leuconostoc mesenteroides*. (A) *L. mesenteroides* KIBGE-IB22; (B) *L. mesenteroides* KIBGE-IB22M20.

3.4. FTIR analysis

FTIR was used to investigate the nature of the functional groups of dextran in terms of monomeric units and their linkages. In the current study, the spectral data obtained for dextran produced by both strains exhibited major characteristic peaks which are distinctive for α-(1 → 6) linkage in dextran (Purama et al., 2009; Seymour, Julian, Jeanes, & Lamberts, 1980; Shingel, 2002; Wang et al., 2007). The spectrum of dextran was studied in the region between 600 cm^{-1} and 4000 cm^{-1} and peaks obtained in the region of 3430 cm^{-1}, 2929 cm^{-1}, 1635 cm^{-1}, 910 cm^{-1}, 1154 cm^{-1}, 1107 cm^{-1} and 1014 cm^{-1} represents the presence for the hydroxyl stretching vibration of a polysaccharide, C–H stretching vibration, carboxyl group, α-glycosidic bond, covalent vibration of C—O—C bond and glycosidic bridge, vibration of C—O bond at C-4 position of D-glucose and chain flexibility around the α-(1 → 6) glycosidic bond, respectively. Dextran produced by both the strains indicated the presence of a linear glucose chain in α-(1 → 6) glycosidic bond formation. Along with these peaks, three more characteristic peaks at 928.90, 846.34 and 820.86 cm^{-1} region were also detected indicating the presence of a branched chain in α-(1 → 3) glycosidic bond formation. It was reported that peaks at 928.90, 846.34 and 820.86 cm^{-1} regions are specifically distinctive for α-(1 → 3) glycosidic bond (Seymour et al., 1980; Wang et al., 2007). Further analysis and reconfirmation of these linkages were accomplished using NMR spectroscopic technique.

3.5. NMR spectroscopic analysis

Structural analysis of dextran can be studied by a number of methods and among them NMR spectroscopy is the most important technique for attaining detailed structural information of dextran through the application of 1D and 2D NMR spectra (^{1}H–^{1}H TOCSY, ^{1}H–^{1}H NOESY, ^{1}H–^{1}H ROESY, ^{13}C–^{1}H HSQC, and ^{13}C–^{1}H HMBC) (Uzochukwu, Balogh, Loefler, & Ngoddy, 2001; Van Leeuwen et al., 2008).

The ^{1}H NMR spectra of dextran from KIBGE-IB22 and KIBGE-IB22M20 are shown in Fig. 3. The anomeric signal centered at 4.9 ppm is characteristic for a typical α-(1 → 6) glycosidic linkage and was observed in both dextran samples along with another anomeric proton signal at 5.27 ppm which is due to the presence of a branch chain typical for α-(1 → 3) linkage. These NMR signals associated for α-(1 → 6) and α-(1 → 3) anomeric proton are similar to the previously reported dextran produced by other strains of *L. mesenteroides* (Bounaix et al., 2009; Maina et al., 2008; Vettori et al., 2012).

The comparison of the chemical shifts showed two different ^{1}H spectra for the dextran produced by KIBGE-IB22 and KIBGE-IB22M20, specifically at the signal region between 3.4 and 4.2 ppm. It was observed that dextran from KIBGE-IB22 expressed more characteristic peaks and were much more complicated in this region as compared to the dextran from KIBGE-IB22M20. According to Bounaix et al. (2009) this bulk region is due to the presence of two exopolysaccharides particularly dextran and levan. Thus the results illustrated in the current study of ^{1}H NMR spectra for the dextran produced by KIBGE-IB22 shows crowded signals of another carbohydrate in this narrow region indicating that the sample from the wild type may contain many similar sugar residues. Various research groups have reported diverse structural peaks for different exopolysaccharides either due to poor isolation and purification procedures or due to the production of more than one type of exopolysaccharide by the same bacterial strain (De Vuyst & Degeest, 1999). Therefore, any explanation on this basis could lead toward false interpretation of the current data obtained for dextran produced by both strains. For further confirmation, dextran from KIBGE-IB22 and KIBGE-IB22M20 were analyzed for ^{13}C NMR chemical shifts (Table 2). Chemical shifts of exopolysaccharides (dextran and levan) produced by both strains were compared with the different strains of lactic acid bacteria. On the basis of ^{13}C NMR spectrum of dextran from KIBGE-IB22, it is strongly suggested that the sample is a mixture of two different exopolysaccharides i.e. dextran and levan; whereas in the case of dextran from KIBGE-IB22M20, there was no contamination of levan found.

A combination of homo and hetero-nuclear 2D NMR experiments were executed in order to assign all the spin systems and to define a specific saccharidic sequence. The anomeric configuration of each monosaccharide unit was assigned on the basis of the $^{3}J_{H\text{-}1,H\text{-}2}$ coupling constant values obtained by DQF-COSY, whereas the values of the vicinal $^{3}J_{H,H}$ ring coupling constants allowed the identification of the relative configuration of each sugar.

Spin system-A was identified as *gluco*-configured sugar residue, as indicated by the large $^{3}J_{H,H}$ ring coupling constants and the chemical shift values, in agreement with *gluco*-configuration of pyranose rings in a $^{4}C_{1}$ conformation (Table 2). In particular, the α-configuration was assigned by the $^{3}J_{H1,H2}$ values (3.1 Hz) and were also confirmed by the *intra*-residual NOE contact of H-1 with H-2 and by H-5/C-5 chemical shift values (Table 2 and Fig. 4A–D). The analysis of scalar correlations in the 2D NMR spectra allowed the identification of the nature of the polysaccharide. The down-field shift of carbon resonances identified the glycosylated positions at O-6 of spin systems-A. The long range correlations present in the HSQC spectrum (Fig. 4G) and HSQC + HMBC spectrum (Fig. 4H) further confirm the nature of dextran of the polysaccharide, constituted by a homopolymer of α-(1 → 6)-Glc.

Whereas spin systems-B was identified as a β-(2 → 6)-fructan, namely levan, as also indicated by the ^{13}C and ^{1}H chemical shift values (Table 2) and by the scalar correlations present in the HSQC spectrum (Fig. 4E) and HSQC + HMBC spectrum (Fig. 4F). The presence of carbon resonances above 80 ppm testified the existence of a sugar residue in furanose form; the carbon resonance at 104.2 ppm of C-2 was an indication of a ketosidic sugar and the $^{3}J_{H,H}$ as well as chemical shift values were also indicative of fructose. The down-field shift of C-6 testified the presence of levan, namely β-(2 → 6)-fructan.

Table 2
The assignments of ^{13}C NMR chemical shifts values for dextran and levan.

Carbon atom	Levan				Dextran			
	Current study		*Leuconostoc mesenteroides* NRRL B512F	*Leuconostoc mesenteroides* ATCC 8293[a]	Current study		*Leuconostoc mesenteroides* CMG713[b]	*Leuconostoc mesenteroides* NRRL B512F[c]
	KIBGE-IB22	KIBGE-IB22M20			KIBGE-IB22	KIBGE-IB22M20		
C-1	60.1	NP	60.1	60.03	97.2	97.2	100.56	98.53
C-2	104.2	NP	104.3	104.34	71.1	71.1	74.25	72.23
C-3	76.2	NP	76.5	76.44	73.2	73.2	76.25	74.23
C-4	75.4	NP	75.4	75.34	70.0	70.0	73.04	71.03
C-5	80.8	NP	80.5	80.44	69.8	69.8	72.43	70.35
C-6	63.6	NP	63.6	63.54	66.8	66.8	68.48	66.35

NP: no production
[a] Olvera et al. (2007).
[b] Sarwat et al. (2008).
[c] Bounaix et al. (2009).

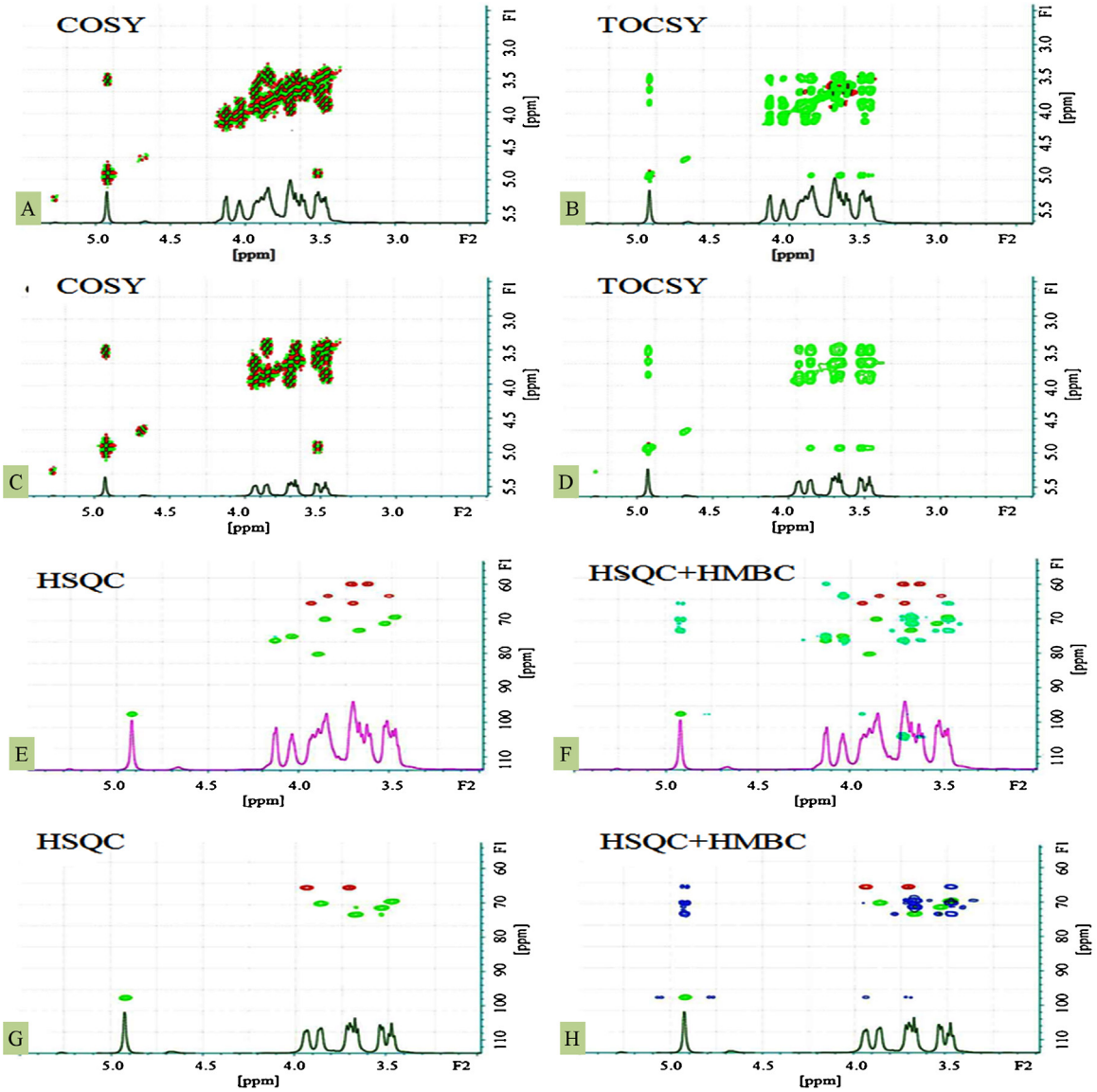

Fig. 4. NMR spectroscopic analysis of dextran and levan. (A) DQF-COSY; (B) TCOSY spectra of dextran from *L. mesenteroides* KIBGE-IB22. (C) DQF-COSY; (D) TOCSY spectra of exopolysaccharide extracted from *L. mesenteroides* KIBGE-IB22M20. (E) HSQC; (F) HSQC + HMBC spectra of dextran produced by *L. mesenteroides* KIBGE-IB22. (G) HSQC; (H) HSQC + HMBC spectra of dextran from *L. mesenteroides* KIBGE-IB22M20.

Based on the NMR spectroscopy data, it was deduced that the dextran produced by KIBGE-IB22 (wild) and KIBGE-IB22M20 (mutant), consist of a linear glucose chain in α-(1 → 6) linkage along with a small proportion of α-(1 → 3) branching. *L. mesenteroides* KIBGE-IB22M20 is a UV derived mutant of indigenously isolated *L. mesenteroides* KIBGE-IB22 (Siddiqui et al., 2013). It was previously noticed that the percent yield of dextransucrase, an enzyme responsible for dextran production, increased 6.75 times after UV exposure and mutant was capable of producing more dextransucrase as compared to the wild type. This remarkable increase in the enzyme production would have led toward higher utilization of sucrose by KIBGE-IB22M20 (15.0%) as compared to KIBGE-IB22 (10.0%). This increase in the utilization of substrate also reduced the fermentation time required for maximum dextran production by KIBGE-IB22M20 (12 h) and which was 24 h in case of KIBGE-IB22. All these factors finally contributed in the increased dextran production by KIBGE-IB22M20 as compared to KIBGE-IB22. Therefore, it is suggested that any increase in the enzyme yield is directly proportional to the utilization of high concentration of substrate and also directly proportional to the degree of polymerization of free glucose to the growing chain of dextran. It was reported earlier that the molecular weight of synthesized dextran is directly proportional to the concentration of sucrose and inversely proportional to the concentration of enzyme (Falconer, Mukerjea, & Robyt, 2011; Kim, Robyt, Lee, Lee, & Kim, 2003). Another feature noticed previously was the presence of two glycoprotein bands of approximately 70–90 kDa in partially purified dextransucrase produced by KIBGE-IB22 using periodic acid-Schiff's staining and after mutation only one single glycoprotein band of dextransucrase was found in KIBGE-IB22M20 dextran sample. Therefore it might be concluded that one of the extra glycoprotein band is of levansucrase. These results were supported by NMR spectroscopic analysis of the polysaccharide produced by KIBGE-IB22 which showed two peaks; one of dextran and another is levan. Whereas levan was not detected in the exopolysaccharide sample produced by KIBGE-IB22M20. It has been reported that some strains of lactic acid bacteria are capable of producing a mixture of exopolysaccahrides including glucans (dextran) and fructan (levan) (Bounaix et al., 2009; Kang, Kimura, & Kim, 2011; Olvera, Centeno-Leija, & Lopez-Munguía, 2007; Shukla et al., 2011; Van Geel-Schutten et al., 1999) but no data is available about the suppression of production of any one of the exopolysaccharide. However, Van Geel-Schutten et al. (1999) has suggested that if pH of the medium is changed, levan production can be suppressed. So far there was no evidence found about the suppression of levan production after the strain was exposed to UV irradiation for a specific time period. In the current study, UV irradiation not only enhanced the dextran production and also suppressed the levan production thus indirectly increased the availability of sucrose for the desired enzyme to produce dextran. Although the exact reason was not known which have caused the suppression of levan production after mutation but it was found that the mutant was capable of producing exclusively dextran only without any concomitant production of levan. It was also suggested that UV radiation might help to suppress the gene responsible for production of levan and resulting in the production of dextran only. An in-depth investigation on the changes at any genetic level by exposure of UV radiation is underway and complete sequences of dextransucrase and levansucrase genes from KIBGE-IB22M20 will be studied to conclude the exact mechanism for this reason.

4. Conclusions

Current study suggested that *L. mesenteroides* KIBGE-IB22M20, a mutant previously generated by UV irradiation, is a most promising strain as it facilitates high percent conversion of substrate into the dextran in less fermentation time as compared to the parent strain (*L. mesenteroides* KIBGE-IB22). In addition, KIBGE-IB22M20 also does not produce any contaminating levan along with dextran and thus can make the downstream process for dextran production more feasible and cost effective.

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