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Characterization of high molecular weight dextran produced by *Weissella cibaria* CMGDEX3

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ABSTRACT

Exopolysaccharide (EPS) producing *Weissella cibaria* CMGDEX3 was isolated from cabbage on sucrose containing De Man, Rogosa and Sharpe (MRS) agar. Dextransucrase activity and dextran yield was found to be 7.1 DSU ml^{-1} and 2.4 g dl^{-1}, respectively. The structural characterization of purified EPS determined by FTIR, ^{1}H and ^{13}C NMR spectroscopy demonstrated that *W. cibaria* CMGDEX3 synthesized a linear dextran that predominately had α (1→6) glycosidic linkages with only a few (3.4%) α (1→3) linked branches. Molecular mass determination showed that it was a high molecular weight dextran of an average >2,000,000 Da. According to our knowledge this is the first report on isolation of dextran synthesizing *Weissella* genus from Pakistan.

1. Introduction

Discovery of microbial origin of cane sugar syrups gelification in 1861 led to the designation of corresponding product as dextran in 1874. The microorganism responsible for the gelification was isolated in 1878 and given the name *Leuconostoc mesenteroides* (Monsan et al., 2001). Dextran is a polysaccharide composed of D-glucose units and features substantial number (at least 50%) of consecutive α (1→6) glycosidic linkage in the main chain and α (1→2), α (1→3) or α (1→4) branch glycosidic linkages (Bounaix et al., 2010; Kim, Robyt, Lee, Lee, & Kim, 2003; Maina, Tenkanen, Maaheimo, Juvonen, & Virkki, 2008; Monsan et al., 2001). Dextrans vary in their type and degree of branching, length of branch chains, spatial arrangement and molecular weight (Maina et al., 2008). Due to the potential of dextran for commercial, nutritional and health applications, it is widely used in chemical, food and pharmaceutical industries (Di Cagno et al., 2006; Galle, Schwab, Arendt, & Ganzle, 2010; Maina et al., 2008; Maina, Virrki, Pyonnonen, Maaheimo, & Tenkanen, 2011; Sarwat, Qader, Aman, & Ahmed, 2008).

Various lactic acid bacteria of the genera *Leuconostoc*, *Lactobacillus*, *Streptococcus* and *Weissella* are known to synthesize extracellular dextran (Bounaix et al., 2010; Shukla & Goyal, 2011). Primarily dextrans are produced by *Leuconostoc* (Maina et al., 2008) and most of the studies have been done on commercial production and structural analysis of dextrans from *Leuconostoc* species particularly strains of *L. mesenteroides* (Maina et al., 2008; Purama, Goswami, Khan, & Goyal, 2009). Commercial production and use in the biochemical and pharmaceutical industry of dextrans by *L. mesenteroides* has been carried out for more than 50 years (Alsop, 1983; Sutherland, 1996) and the most widely used dextran is produced by *L. mesenteroides* B512F which is a linear dextran with around 5% α (1→3) linked branches (Maina et al., 2011; Monsan et al., 2001; Purama et al., 2009).

Attention to dextran synthesized by *Weissella* has been given in the last decade (Bounaix et al., 2009, 2010; Di Cagno et al., 2006; Galle et al., 2010; Kang, Chung, Kim, Yang, & Oh, 2006; Kang, Oh, & Kim, 2009; Katina et al., 2009; Maina et al., 2008, 2011; Shukla & Goyal, 2011). The genus *Weissella* was proposed in 1993 during a study of *Leuconostoc* like microorganisms (Collins, Samelis, Metaxopoulos, & Wallbanks, 1993). *Weissella* is phylogenetically related to *Leuconostoc* and *Oenococcus* and has risen from the reclassification of *L. paramesenteroides* and some related "atypical" hetero-fermentative *Lactobacilli* (Bounaix et al., 2010). *Weissella* strains have been isolated from a variety of sources such as spring water, meat, raw milk, fresh vegetables, sugar cane, carrot juice, soya, kimchi and sourdough (Bjorkroth et al., 2002; Galle et al., 2010; Maina et al., 2011; Shukla & Goyal, 2011).

Weissella cibaria which is a Gram-positive, rod shape, obligate heterofermentative bacillus (Bounaix et al., 2010; Kang et al., 2009) was first isolated by Bjorkroth et al. in 2002. Though production of slime (dextran) by *W. cibaria* was observed on sucrose containing agar in the study but it was stated under the

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phenotypic characterization. Dextran production has typically served as a phenotypic test in the identification of bacteria classified in the genus *Weissella* (Bounaix et al., 2010; Maina et al., 2008). The synthesis and detailed study of EPS by *W. cibaria* was first reported in 2006 by Di Cagno et al. In the same year Kang et al. also reported production of water soluble glucan from sucrose by *W. cibaria* which was a linear dextran with only α (1→6) glycosidic linkages. The presumption of dextran structure based on enzymatic degradation for *W. cibaria* strains was first reported in 2006 (Kang et al., 2006) and 2008 (Schwab, Mastrangelo, Corsetti, & Gänzle, 2008) while first structural description of dextrans from *Weissella* species (*Weissella confusa* E392) was given in 2008 (Bounaix et al., 2010; Maina et al., 2008). *W. confusa* E392 showed better growth, higher EPS production and more linear dextran than *Leuconostoc citreum* E497 and conventional *L. mesenteroides* B512F. Bounaix et al., in 2009 reported structural description of dextran from five strains of *W. cibaria* and one strain of *W. confusa* which also showed few (2.4–3.3%) α (1→3) branch linkages. Highly linear linkage pattern in *Weissella* species indicates that this may be a common feature of *Weissella* species (Maina et al., 2011). For the first time constitutive dextransucrase activity without sucrose induction was reported in *W. cibaria* and *W. confusa* strains which is so far known in *Streptococcus* sp. and some *Lactobacillus* strains for glucansucrases (Bounaix et al., 2010). Shukla and Goyal (2011) reported a novel high glucan producing *W. confusa* which exhibited higher glucansucrase activity and glucan concentration than conventional *L. mesenteroides* B640 and *L. mesenteroides* B512F. Hence it is highly valuable to isolate more *Weissella* specie strains and explore their potential to synthesize dextrans. In the present study a dextran synthesizing bacterial strain *W. cibaria* CMGDEX3 was isolated and the extracted dextran was characterized.

2. Experimental

2.1. Isolation and purification of dextran producing bacteria

For isolation of dextran synthesizing bacteria, De Man, Rogosa and Sharpe (MRS medium) containing 15% sucrose (MRS-S) was used. Sample of cabbage purchased from local market was inoculated in MRS-S and incubated at 30 °C in static condition. After 48 h of incubation culture was streaked on MRS-S agar plate and incubated at 30 °C for 24 h. Mucoid colonies exhibiting slime production on agar were selected, purified and characterized for colonial and cellular morphology on MRS agar.

2.2. 16S rRNA identification

16S rRNA gene of CMGDEX3 was amplified using universal primers 518F (CCAGCAGCCGCGGTAATACG) and 800R (TACCAGGGTATCTAATCC) and commercially sequenced by Macrogen, Korea. Sequence obtained was submitted to GenBank and searched for similarity using the BLAST tool on web page of NCBI.

16S rRNA sequences of all dextran producing bacterial strains reported from Pakistan and dextran or glucan producing *Weissella* and *Leuconostoc* species were retrieved from GenBank and phylogenetic tree was constructed in MEGA 5 using the neighbour-joining method after alignment in Clustal W program.

2.3. Production of EPS and enzyme assay

Production of EPS by CMGDEX3 was studied in medium containing ($g\,l^{-1}$): sucrose 150.0, bactopeptone 5.0, yeast extract 5.0, K_2HPO_4 15.0, $MnCl_2$ 0.01, NaCl 0.01, $CaCl_2$ 0.05 and pH was adjusted to 7.0 before sterilization. CMGDEX3 was inoculated in 10 ml medium and incubated at 25 °C for 24 h without shaking. 24 h grown culture was transferred into 90 ml fresh medium of same composition and incubated on same parameters. After 24 h, third transfer was carried out in 900 ml medium of same composition and incubated on same parameters. For determination of dextransucrase production, culture was incubated for 8 h at 25 °C with 25 $g\,l^{-1}$ sucrose concentration. Dextransucrase activity was determined by measuring the reducing sugar (Aman, Siddiqui, & Qadar, 2012).

2.4. EPS extraction and purification

EPS from culture medium was precipitated with chilled ethanol as described by Qader, Iqbal, Aman, Shireen, and Azhar (2006) with slight modification. Before vacuum drying, extracted EPS was washed with distilled water and precipitated with chilled ethanol three times. Precipitated EPS from CMGDEX3 was purified by dialysis using a membrane (with a nominal cut off value M_r 8000–12,000 Da) and lyophilized before subjecting to various analyses for characterization. For calculating the yield, dextran was lyophilized instead of vacuum drying.

2.5. FTIR, NMR and GPC analysis of purified EPS

The Fourier-Transform infrared (FTIR) analysis of the purified EPS was recorded using spectrometer NICOLET AVATAR 370 DTGS Smart Omni sampler (Thermo Electron Corporation) interfaced with EZ Omnic software. The ^1H NMR was recorded on Bruker AM 300 and ^{13}C NMR spectra were recorded on Bruker 75.4 in D_2O. The average molecular weight of the purified EPS was determined by gel permeation chromatography (GPC) on LKB gel filtration system using Blue dextran 2000 as standard (Qader et al., 2006).

3. Results and discussion

A Gram-positive coccobacilli bacterial strain CMGDEX3 was isolated from cabbage on MRS-S agar. Approximately full length sequence of 16S rRNA gene of CMGDEX3 (GenBank ID: HQ909767) was amplified which exhibited 99% homology with *W. cibaria* in BLAST. Phylogenetic tree (Fig. 1) showed the position of *W. cibaria* CMGDEX3 among other dextran or glucan producers. *W. cibaria* CMGDEX3 lied with other reported dextran producing *Weissella* species whereas *Leuconostoc* species particularly all reported dextran producing *Leuconostc* species from Pakistan were distantly placed from *W. cibaria* CMGDEX3 in the tree.

W. cibaria CMGDEX3 exhibited highly viscous slimy growth on MRS-S agar within 24 h of incubation. Mucoid bacterial colonies and slime production on sugar added agar medium are characteristics of EPS producing bacteria (Bounaix et al., 2009; Milintawisamai, Naimsanit, Ngasan, Pliansinchai, & Weerathaworn, 2009; Tallgren et al., 1999; Vijayendra, Palanivel, Mahadevamma, & Tharanathan, 2009). Dextransucrase activity detected in *W. cibaria* CMGDEX3 was 7.1 DSU ml^{-1} in static condition. EPS extracted from liquid culture medium was water soluble, white and fluffy. Yield of the extracted EPS from *W. cibaria* CMGDEX3 was 2.4 g dl^{-1} within 24 h of incubation. Most of the studies on production of dextran by species of *Weissella* have been carried out in situ in sourdough (Shukla & Goyal, 2011). *W. cibaria* 10 M was reported to produce >60 g of isomaltooligosaccharides kg^{-1} DM and 0.6 g of dextran kg^{-1} DM in sorghum sourdough (Schwab et al., 2008). Significant production, 11–16 g kg^{-1} DW, of dextran in wheat sourdough by *W. confusa* VTTE-90392 was reported for the first time by Katina et al. (2009). In another study, *Weissella* strains were reported to produce 0.8–8 g kg^{-1} EPS and gluco-oliosaccharides in wheat and sorghum sourdough (Galle et al., 2010). *W. confusa* E392 was reported to

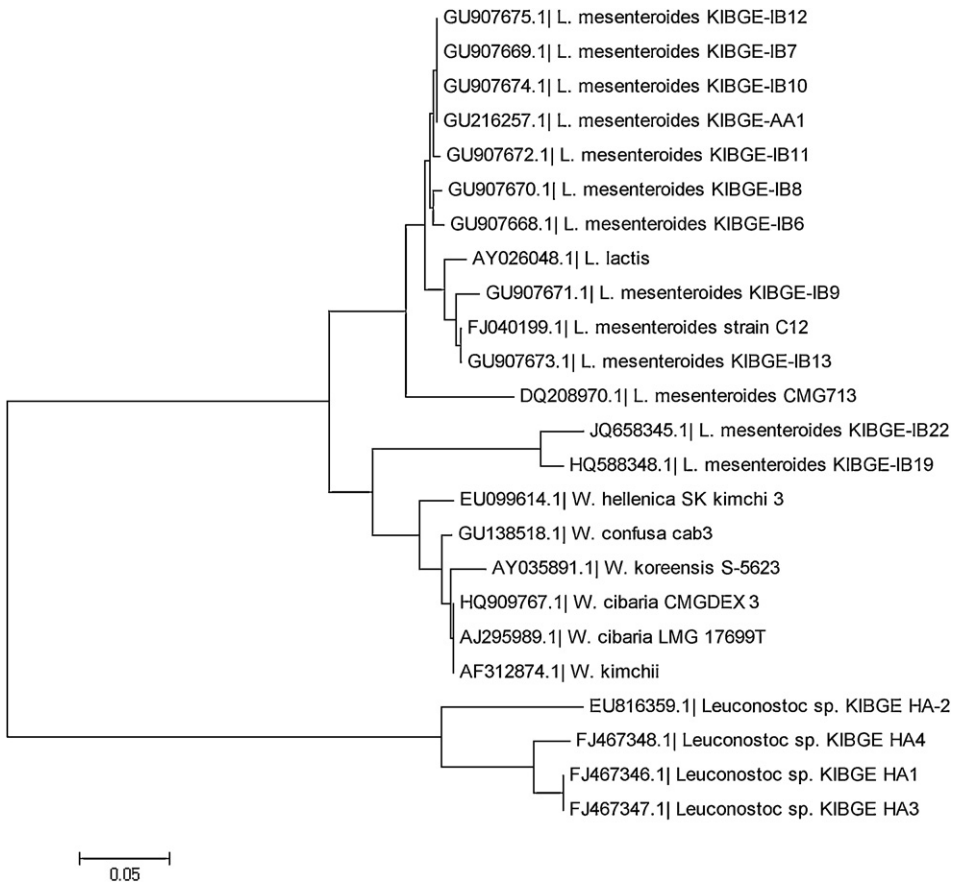

Fig. 1. Neighbor-joining phylogenetic tree based on homologies of 16S rRNA sequences of *W. cibaria* CMGDEX3 with other dextran or glucan producing *Weissella* and *Leuconostoc* species.

produce higher yield of dextran in comparison with *L. citreum* E497 and *L. mesenteroides* B-512F (Maina et al., 2008). In another study (Shukla & Goyal, 2011) glucan-hyperproducing *W. confusa* produced 34 mg ml^{-1} of glucan within 12 h of incubation which was very high yield in comparison with other reported lactic acid bacteria.

3.1. FTIR spectrum

FTIR spectrum of the purified EPS from *W. cibaria* CMGDEX3 (Fig. 2) exhibited close similarity with dextran of *L. mesenteroides* B640 which is a water soluble, highly linear dextran with consecutive α (1→6) linkages without any branching (Purama et al., 2009). In spectrum of the dextran from *L. mesenteroides* B640 the absorption peak which indicated the existence of α-glycosidic bond at 906 cm^{-1} while the main characteristic bands were found at 1154, 1103 and 1020 cm^{-1} (Purama et al., 2009). In the spectrum of EPS from *W. cibaria* CMGDEX3 the absorption peak was at 908 cm^{-1} while the main characteristic bands were found at 1151, 1105 and 1019 cm^{-1}. Purama et al. (2009) reported the band at 1154 cm^{-1} due to valent vibration of C—O—C bond and glycosidic bridge, band at 1103 cm^{-1} due to vibration of the C—O bond at the C-4 position of glucose residue and the band at 1020 cm^{-1} due to the great chain flexibility present in dextran around α (1→6) glycosidic bonds. In different studies the band due to hydroxyl stretching vibration of the polysaccharide was observed in the region of 3400 cm^{-1} (Liu et al., 2007), 3434 cm^{-1} (Purama et al., 2009) and 3424 cm^{-1} (this study). The bands due to C—H stretching vibration and carboxyl group, respectively were in the region of 2930 cm^{-1} and 1639 cm^{-1} (Liu et al., 2007), 2928 cm^{-1} and 1639 cm^{-1} (Purama et al., 2009), 2935 cm^{-1} and 1641 cm^{-1} (this study).

3.2. ^{1}H NMR analysis

The ^{1}H NMR spectrum of EPS from *W. cibaria* CMGDEX3 (Fig. 3) also resembled to dextran from *L. mesenteroides* B640 (Table 1). Spectral resonances of EPS from *W. cibaria* CMGDEX3 were observed in the region of 3.54–4.98 ppm. For different dextrans the distribution of ^{1}H NMR resonances are reported in 3–6 ppm (Seymour, 1979a). Various reported dextrans have shown ^{1}H NMR spectral resonances (H-2, H-3, H-4, H-5 and H-6) in 3–4 ppm region and the hemiacetal H-1 resonance in 4–6 ppm region (Sidebotham, 1974). EPS from *W. cibaria* CMGDEX3 showed the resonance at 4.98 ppm which is a reported typical dextran α (1→6) chain-extending anomeric signal (Maina et al., 2008). In different reported dextrans the anomeric signal was found at 4.95 ppm (Seymour, 1979b), 4.96 ppm (Seymour, 1979a) and 4.98 ppm (Bounaix et al., 2009; Maina et al., 2008; Purama et al., 2009). Dextran of *W. cibaria* CMGDEX3 also showed an additional low intensity anomeric signal at 5.32 ppm which was attributed to the presence of α (1→3) linked branches. The percentage of α (1→3) linkage was calculated 3.4% from relative intensities of the

Table 1
^{1}H NMR chemical shifts of dextran from *W. cibaria* CMGDEX3 exhibited close resemblance with dextran produced from *L. mesenteroides* B640.

Bacterial strain	H-1	H-2	H-3	H-4	H-5	H-6
W. cibaria CMGDEX3	4.98	3.58	3.73	3.54	3.93	3.99
L. mesenteroides B640	4.98	3.58	3.73	3.54	3.92	3.99

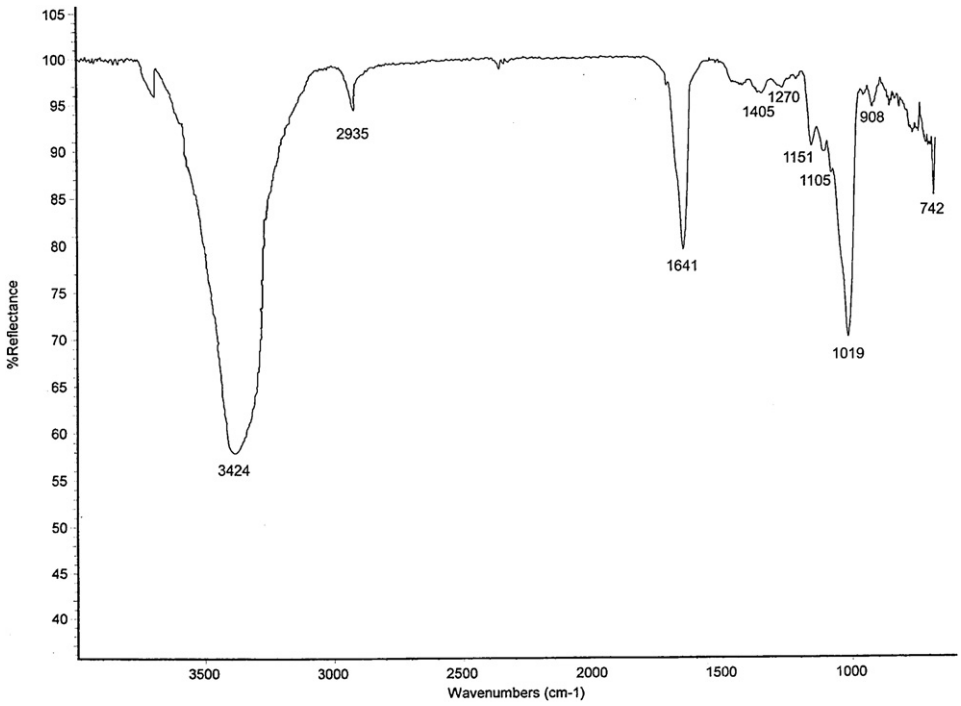

Fig. 2. FTIR spectrum of purified dextran from *W. cibaria* CMGDEX3.

anomeric signals. Anomeric signals between 4.9 and 5.3 ppm are reported to be due to branching in dextrans (Bounaix et al., 2009; Maina et al., 2008; Seymour, 1979a). Dextran from *W. confusa* E392 and *L. mesenteroides* B512F exhibited only one additional low intensity anomeric signal at 5.32 ppm which was reported as α (1→3) branching. Dextran from *L. citreum* E497 exhibited two additional intense anomeric signals at 5.11 and 5.18 ppm and a low intensity anomeric signals at 5.32 ppm. The signals at 5.11 and 5.3 ppm were attributed to α (1→2) and α (1→3) linked branches, respectively (Maina et al., 2008). In a study of Bounaix et al. (2009) dextran from different bacterial strains exhibited anomeric proton at 4.98, 5.11 and 5.32 ppm which were attributed to α (1→6), α (1→2) and α (1→3) linkage, respectively. Dextran from *L. mesenteroides* B1355 also showed the resonance peak at 5.3 ppm which indicated branch linkages (Seymour, 1979b). Presence of no other signal in the region of 4.9–5.3 ppm, except 5.32 ppm, indicates the absence of any other branching than α (1→3) in dextran from *W. cibaria* CMGDEX3. Previously dextrans extracted from species of *Weissella* belonging to *cibaria* and *confusa* have been reported as linear dextrans containing only α (1→6) linkages (Kang et al., 2006) or with few (2.4–3.3%)

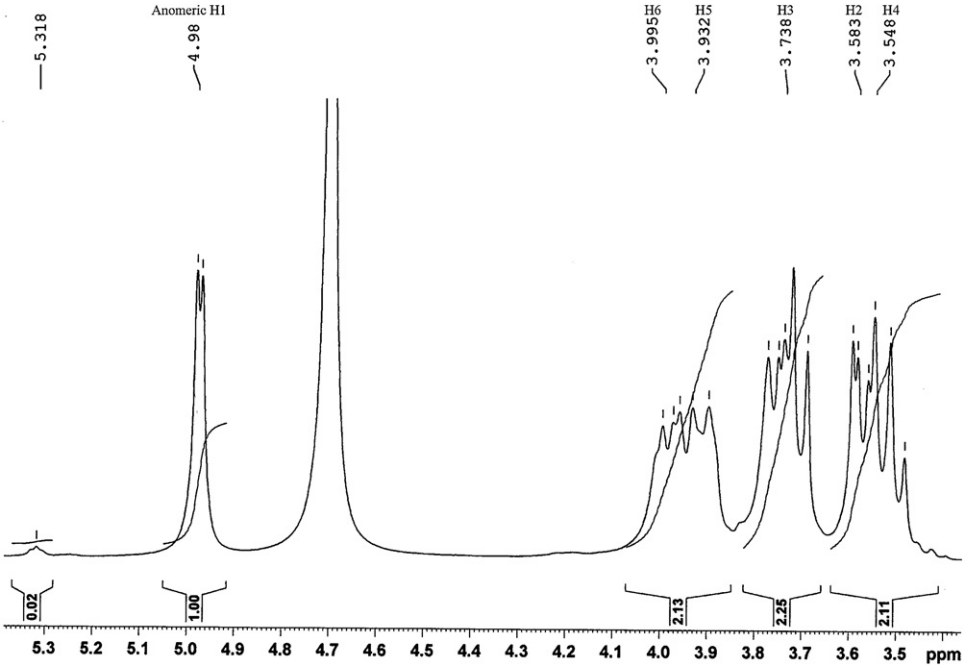

Fig. 3. ^{1}H NMR (300 MHz, D_2O) spectrum of purified dextran from *W. cibaria* CMGDEX3.

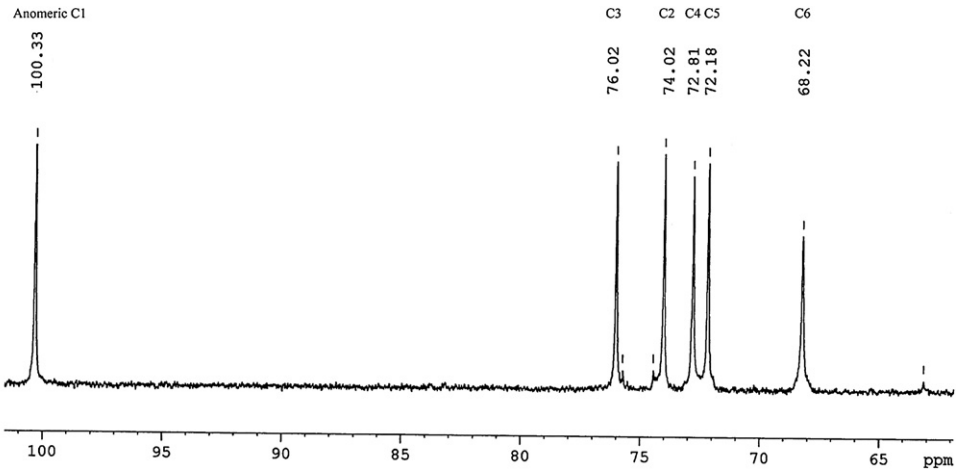

Fig. 4. ^{13}C NMR (75.4 MHz, D_2O) spectrum of purified dextran from *W. cibaria* CMGDEX3.

α (1→3) linked branches (Bounaix et al., 2009) or 2.7% α (1→3) linked branches (Maina et al., 2008).

3.3. ^{13}C NMR analysis

Dextrans typically have the ^{13}C anomeric signals downfield at ~90 ppm while C-2, C-3, C-4 and C-5 appear in the 70–75 ppm region and C-6 is normally upfield at ~60 ppm (Maina et al., 2008). Seymour (1979b) studied structure of a series of dextrans by ^{13}C NMR and reported the anomeric region at 95–105 ppm and 70–75 ppm region associated with free positions at C-2, C-3 and C-4. The ^{13}C NMR spectrum of EPS from *W. cibaria* CMGDEX3 showed the major resonance in the anomeric region at 100.3 ppm and C-6 resonance occurred at 68.2 ppm (Fig. 4). Following the reported pattern (Purama et al., 2009; Uzochukwu, Balogh, Loefler, & Ngoddy, 2002) approximately equal intensities of peaks at C-1 and C-6 indicated presence of α (1→6) glycosidic bond in dextran of *W. cibaria* CMGDEX3. Seymour (1979b) reported 75–85 ppm region for dextrans branched at C-2, C-3 or C-4. Resonance at 76.0 ppm region in the ^{13}C NMR spectrum of dextran from *W. cibaria* CMGDEX3 indicated branch linkage at C-3 which was also supported by resonance at 5.32 ppm in its ^{1}H NMR spectrum. ^{13}C NMR spectrum of dextran from *W. cibaria* CMGDEX3 exhibited close resemblance to dextran from *L. mesenteroides* CMG713 (Sarwat et al., 2008) and observed values for C-1 to C-6 of dextran from *W. cibaria* CMGDEX3 showed no significant difference with the reported values (Table 2).

3.4. Average molecular weight

An average molecular weight of dextran from *W. cibaria* CMGDEX3 was found to be >2,000,000 Da by GPC. Bounaix et al. (2009) reported production of glucan greater than 10^6 Da from *W. cibaria* and *W. confusa*. In 2008 glucan of 203,000 molecular mass from *W. hellenica SKkimchi* 3 was reported (Kim, Seo, Hwang, Lee, & Park, 2008). Higher molecular weight dextran with few branch linkages is considered a good quality dextran (Maina et al., 2011). Use of high molecular weight microbial dextran with low degree of branching is preferable in sourdough baking to produce good quality bread. The required molecular weight has been reported to be from 2×10^6 to about 4×10^6 Da (Katina et al., 2009). Some studies have already pointed out that EPS from *W. cibaria* improves the textural properties of bread (Di Cagno et al., 2006; Galle et al., 2012; Katina et al., 2009; Schwab et al., 2008) and *Weissella* strains are suitable candidates to improve the quality of conventional and gluten free bread (Galle et al., 2010). *W. cibaria* 10 M is reported to produce probiotics during bread making process (Schwab et al., 2008) while another study has suggested *W. cibaria* as probiotic for application in oral health due to its potential to inhibit *Streptococcus mutans* biofilm formation in vitro and in vivo (Kang et al., 2006).

Table 2
^{13}C NMR chemical shifts of dextran from *W. cibaria* CMGDEX3 exhibited resemblance with dextran produced from *L. mesenteroides* CMG713.

Bacterial strain	C-1	C-2	C-3	C-4	C-5	C-6
W. cibaria CMGDEX3	100.33	74.02	76.02	72.81	72.18	68.22
L. mesenteroides CMG713	100.56	74.25	76.25	73.04	72.43	68.48

4. Conclusion

Dextran producing *Weissella* strains have promising applications in several sectors (Bounaix et al., 2010). In the present study dextran producing bacterial strain *W. cibaria* CMGDEX3 was isolated. The results of GPC, FTIR, ^{1}H NMR and ^{13}C NMR analysis confirmed that dextran from *W. cibaria* CMGDEX3 is a high molecular weight, linear dextran with predominant α-(1→6) linkages and few (3.4%) α-(1→3) linked branches. High molecular weight linear dextran of *W. cibaria* CMGDEX3 can be significant in various industrial applications particularly in sourdough baking. According to our knowledge this is the first report on isolation of dextran synthesizing *W. cibaria* from Pakistan. Most of the studies in this region have been conducted on glucansucrase and dextran from *Leuconostoc* species (Aman, Qadar, Bano, & Azhar, 2009; Aman et al., 2012; Qader, Iqbal, Rizvi, & Zuberi, 2001; Qader et al., 2006; Qader & Aman, 2012; Sarwat et al., 2008). Constructed phylogenetic tree exhibited the position of *W. cibaria* CMGDEX3 at distance from those *Leuconostoc* species which shows that the isolated strain is at distant from studied dextran producers of this region in evolutionary point. Hence it is important to explore potential of microflora other than *Leuconostoc* of this region for the production of industrially valuable dextrans. Optimization of dextran yield from *W. cibaria* CMGDEX3 is in progress which is another important aspect to explore worth of bacterial strain in industrial application. High linearity of the dextran from *W. cibaria* CMGDEX3 strengthens the proposed idea that high linearity may be the common feature of *Weissella* species and supports the substitution of conventional dextran usage in different industries.

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