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Dextran synthesized by *Leuconostoc mesenteroides* BD1710 in tomato juice supplemented with sucrose

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ABSTRACT

The characteristics of the growth of *Leuconostoc mesenteroides* BD1710 and the synthesis of dextran in tomato juice supplemented with 15% sucrose were assayed. *L. mesenteroides* BD1710 could synthesize approximately 32 g L^{-1} dextran in the tomato-juice–sucrose medium when cultured at 28 °C for 48 h, which was on the same level as the dextran yield in a chemically defined medium. The viscosity of the cultured tomato-juice–sucrose medium with various dextran contents was also measured. The results of the monosaccharide composition, molecular-weight distribution, Fourier transform infrared spectra (FTIR) and nuclear magnetic resonance spectra (NMR) showed that the polysaccharide synthesized by *L. mesenteroides* BD1710 in the tomato-juice–sucrose medium was dextran with a peak molecular weight of 6.35×10^5 Da, a linear backbone composed of consecutive α-(1 → 6)-linked D-glucopyranosyl units and approximately 6% α-(1 → 3) branches.

1. Introduction

Glucan, one of the homopolysaccharides containing only glucose residue, consists of two types, α- and β-glucan. Unlike β-glucan, the synthesis of which does not involve any glucansucrase (E.C. 2.4.1.5; also known as glucosyltransferase or glycansucrase) or sucrose (Duenas-Chasco et al., 1997), α-glucan is biosynthesized from sucrose as the sole substrate by secreted or cell-anchored extracellular glucansucrases (Monsan et al., 2001; van Hijum, Kralj, Ozimek, Dijkhuizen, & van Geel-Schutten, 2006). For the energy required for α-glucan synthesis derived from the hydrolysis of the glycosidic linkage in sucrose, the process could be undertaken without any cofactor (Moulis et al., 2006). According to the principle glycosidic linkages present in backbone of α-glucans, these polymers are classified as (i) dextran with α-(1 → 6) glycosidic linkages (Van Cleve, Schaefer, & Rist, 1956), (ii) mutan with α-(1 → 3) glycosidic linkages (Kralj et al., 2004), (iii) alternan with alternating α-(1 → 6) and α-(1 → 3) glycosidic linkages (Cote & Robyt, 1982), and (iv) reuteran with α-(1 → 4) and α-(1 → 6) glycosidic linkages (Van Geel-Schutten et al., 1999). The dextran from *Leuconostoc mesenteroides* is one of the biopolymers first produced on an industrial scale, and widely applied in the food as well as the nonfood industries, e.g., as a texture modifier in food, as a bioseparation agent (Sephadex® gels), or as a plasma substitute, iron carrier or anticoagulant in pharmacy, etc. (Naessens, Cerdobbel, Soetaert, & Vandamme, 2005). To meet the demand for the manufacturing of *L. mesenteroides* dextran on large scale, several chemically defined mediums (CDM) had been tested (Foucaud, Francois, & Richard, 1997; Kim, Eom, Lee, Han, & Han, 2004; Kim et al., 2012; Tsuchiya et al., 1952). However, whether the chemicals employed in the CDM were associated with potential toxicological risks has been of great concern for consumers. Therefore, the use of a naturally derived medium to produce dextran is a better choice for the food industry.

Tomatoes (*Lycopersicon esculentum*) and tomato-based foods are consumed worldwide for their low contents in fat and calories and rich contents in dietary fibre, lycopene, beta carotene, vitamin C and several trace elements (including selenium, cooper, manganese and zinc) (Parfitt et al., 1994). One particular tomato product, fermented tomato juice by lactic acid bacteria (LAB), is recognized as a probiotic beverage with beneficial properties (Thakur, Singh, & Nelson, 1996). Four LAB species, i.e., *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei* and *Lactobacillus delbrueckii*, had been utilized to ferment tomato juice, and the tomato juice was found to be suitable for producing probiotic beverages for vegetarians as well as consumers allergic to dairy-based products (Yoon, Woodams, & Hang, 2004). Immobilization of *L. acidophilus* could improve the survival of the bacteria in the fermented tomato juice, with the protection effect provided by cell immobilization

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against the unfavourable environmental conditions, such as low pH values (King, Huang, & Tsen, 2007; Tsen, Lin, Huang, & King, 2008). Tomato juice supplemented with some sugars such as fructooligosaccharide (FOS) can promote the fermentation process by *Bifidobacterium* species and improve the quality of fermented tomato juice by reducing off-flavours, while the beneficial lycopene content was not significantly affected (Koh, Kim, & Oh, 2010). Similar phenomena had been also observed in other lactic acid bacteria fermented tomato juice supplemented with honey (Priya, Munishamanna, & Shree, 2013). However, few studies have been reported on the glucan biosynthesis by *L. mesenteroides* in tomato juice supplemented with sucrose.

In the present study, the growth characteristics of *L. mesenteroides* BD1710 (CGMCC6432) in tomato juice supplemented with sucrose (tomato-juice–sucrose medium) were investigated, including the changes of the viable cell counts, pH, viscosity and titratable acidity and the contents of sucrose, fructose, glucose and dextran yields during the cultivation. The dextran yields by *L. mesenteroides* BD1710 in the tomato-juice–sucrose medium and CDM were also compared. Finally, monosaccharide composition analysis, determination of the molecular-weight distribution, FTIR and NMR were performed to characterize the dextran synthesized by *L. mesenteroides* BD1710. This is the first report to elucidate the dextran synthesized in tomato juice supplemented with sucrose by *L. mesenteroides*.

2. Materials and methods

2.1. Bacterial strain, propagation and storage

L. mesenteroides BD1710 (CGMCC6432) was provided by Bright Dairy & Food Co., Ltd., China. The bacterial strain was routinely streaked on M17 agar (Merck, Germany) supplemented with 5% sucrose and incubated at 30 °C aerobically. The strain was stored in M17 broth (Merck, Germany) supplemented with 10% glycerol at −80 °C.

2.2. Preparation of the tomato-juice–sucrose medium and the chemically defined medium (CDM)

Fresh, raw tomatoes were purchased from local market and washed with tap water. The fruits were cut into small cubes and grounded in a pulper and filtered through cotton gauze to remove the majority cake containing peel and seeds. The crude tomato juice was further centrifuged at 15,000 × *g* for 20 min at 4 °C, and the supernatant was collected as tomato juice. The tomato juice was then supplemented with 15% (w/v) sucrose, and the pH value of the mixture was adjusted to 7.0 by adding drops of 5.0 M NaOH. After being sterilized at 121 °C for 20 min and cooling to ambient temperature, the tomato-juice–sucrose medium was utilized for *L. mesenteroides* cultivation.

The CDM (Majumder, Singh, & Goyal, 2009) used in this study was composed of sucrose (150 g L^{-1}), yeast extract (5 g L^{-1}), peptone (10 g L^{-1}), K_2HPO_4 (2 g L^{-1}), $MgSO_4$ (0.2 g L^{-1}), $CaCl_2$ (0.02 g L^{-1}), NaCl (0.01 g L^{-1}), $FeSO_4$ (0.01 g L^{-1}) and $MnSO_4$ (0.01 g L^{-1}), and the pH value was adjusted to 7.0 before sterilization at 121 °C for 20 min.

2.3. Fermentation condition

A loop of fresh culture of *L. mesenteroides* BD1710 on M17 agar supplemented with 5% sucrose was inoculated into 10 mL of sterile M17 broth supplemented with 5% sucrose, and incubated at 28 °C on a rotary shaker of 180 rpm for 24 h. The activation procedure in M17 broth supplemented with 5% sucrose was repeated twice and then 3 mL of the cultured broth was transferred aseptically into 250-mL Erlenmeyer flasks containing either 150 mL of the tomato-juice–sucrose medium or CDM. The fermentation was performed at 28 °C on a shaker at 180 rpm. At 0, 3, 6, 9, 12, 24, and 48 h, 5-mL samples were taken out for further assays.

2.4. Preparation and purification of dextran

The cells in the cultured broth were removed by centrifugation at 15,000 × *g* for 20 min at 4 °C after the broth was diluted by four equivalents of distilled water. Then, four equivalents of pre-chilled ethanol were added to the supernatant, and the mixture was stored overnight at 4 °C. The precipitate was collected by centrifugation at 15,000 × *g* at 4 °C for 20 min and re-dissolved in deionized water. This process was repeated twice to purify the dextran. The dextran yield was determined by weighing the polysaccharide mass after being freeze-dried (Freezone12, Labconco, USA).

The dextran from the tomato-juice–sucrose medium by *L. mesenteroides* BD1710 cultured for 48 h was prepared by the above procedure. Before further assays to characterize the structure of the BD1710 dextran, e.g. analysis of the monosaccharide composition, the molecular-weight distribution, etc., the prepared biopolymer was checked for the residual proteins and free mono- and disaccharides including sucrose, glucose and fructose.

The protein content of the prepared *L. mesenteroides* BD1710 dextran solution of 5 mg mL^{-1} was detected by the Bradford assay using bovine serum albumin (Sigma, St. Louis, MO, USA) as standard (Bradford, 1976), while free mono- and disaccharide contents were assayed by the method listed in Section 2.6.

2.5. Analytical methods

2.5.1. Viable cell counts

The viable cell numbers of *L. mesenteroides* BD1710 in the cultured broths were enumerated by plate counting. Aliquots of the cultured broth samples at 0, 3, 6, 9, 12, 24, and 48 h after 10-fold serial dilution in sterilized physiological saline were plated onto M17 agar containing 5% sucrose, and the plates were incubated aerobically at 28 °C for 48 h. The viable cell counts of *L. mesenteroides* BD1710 were expressed as colony forming units (cfu) per millilitre.

2.5.2. pH, titratable acidity and viscosity

The pH value was measured by a pH-meter (PB-100, Sartorius, GER), and the titratable acidity (TA) of the culture broth was determined with 0.1 M NaOH using 0.5% phenolphthalein as the indicator. The viscosity of the cultured broth was measured by a viscometer (R 180, proRheo, GER) at 25 °C.

2.6. Sucrose, glucose and fructose contents

The sucrose, glucose and fructose contents of the samples at various intervals were determined by the high performance liquid chromatography (HPLC) method described by Villanueva-Suarez, Redondo-Cuenca, Rodriguez-Sevilla, and Martinez (2003).

2.7. Monosaccharide composition

To determine the monosaccharide composition, the lyophilized exopolysaccharide of *L. mesenteroides* BD1710 was dissolved in deionized water at a concentration of 5 mg mL^{-1} and hydrolyzed with 4 M trifluoroacetic acid (TFA) at 110 °C for 2 h. The excess TFA was removed by adding equal volumes of methanol to the digested samples and blowing dry with a nitrogen purge. The hydrolyzed sample was then dissolved in deionized water, adjusted to its initial volume, and filtered through a 0.22-μm membrane (Millipore, MA, USA). The monosaccharides were identified and quantified with a Dionex high-performance anion-exchange chromatography

(HPAEC) system (DX-500) equipped with a pulsed amperometric detector (Sunnyvale, CA, USA). The monosaccharides were eluted by a linear gradient of NaOH solution (100 mM to 300 mM) at a flow rate of 0.5 mL min^{-1}, according to the procedure described by Wood, Weisz, and Blackwell (1994). Rhamnose, arabinose, galactose, glucose, xylose, mannose and fructose (Sigma, USA) were used as references. The percentages of the monosaccharides were calculated from the peak areas using a response factor.

2.8. Molecular-weight distribution

The dextran molecular weight distributions were determined by high-performance size-exclusion chromatography (HPSEC) using a Waters 2690 (Waters, USA) instrument equipped with a Waters 2410 differential refractometer. Two Waters Ultrahydrogel™ linear columns (7.8 × 300 mm) were maintained in series, using an eluent containing 0.1 M of $NaNO_3$ at a flow rate of 0.9 mL min^{-1}. The columns and guard columns were maintained at 40 °C, and the samples were filtered through a 0.22-μm filter (Sartorius, USA) before injection. Commercial dextrans with molecular weights ranging from 1000 to 1400,000 Da (Sigma–Aldrich, USA) were used as standards.

2.9. Fourier transform infrared spectroscopic analysis

The FTIR spectra of BD1710 dextran from the cultured tomato-juice–sucrose medium were obtained using a Nicolet Nexus 470 spectrometer (NICOLET Co., USA) employing KBr disks.

2.10. ^{1}H and ^{13}C NMR spectroscopy analysis

Ten milligrams of BD1710 dextran from the cultured tomato-juice–sucrose medium were dissolved separately in 0.5 mL of pure D_2O and then placed into 5-mm NMR tubes. One-dimensional ^{1}H NMR utilizing tetramethoxysilane as internal standard and ^{13}C NMR spectra were obtained on a Varian Mercury Plus 400 spectrometer (Varian, USA) at 25 °C. The ^{1}H NMR data collection consisted of 256 acquisitions.

2.11. Statistical analysis

All experiments were performed at least in triplicate. Analysis at every time point from each experiment was performed in triplicate. The means, standard errors, and standard deviation were calculated from the replicates with the experiments and analysis using Microsoft Excel 2007 and OriginPro 8.0.

3. Results and discussion

3.1. Viable cell count, pH, titratable acidity and viscosity of the cultured broth

The changes in the viable cell counts and the pH during *L. mesenteroides* BD1710 growth in the tomato-juice–sucrose medium were shown in Fig. 1A. Although the seeded tomato-juice–sucrose medium was with a low initial pH value of 5.72, resulted from the inoculation procedure as well as the damage of some carbohydrates in the medium during the sterilization, *L. mesenteroides* BD1710 could grow well in the medium, with a significant ($P < 0.05$) increase in the viable-cell count from an initial value of approximately 7.5 log_{10} to 9.3 log_{10} after 10 h of cultivation, while a sharp decrease in the pH value from 5.72 to as low as 3.88 at the 48 h of cultivation. The optimum pH of the culture medium of 5.50 for *L. mesenteroides* to biosynthesize dextran had been reported by several other researchers (Lazić, Veljković, Vučetić, & Vrvić, 1993; Santos, Teixeira, & Rodrigues, 2000), which was approximate to the initial

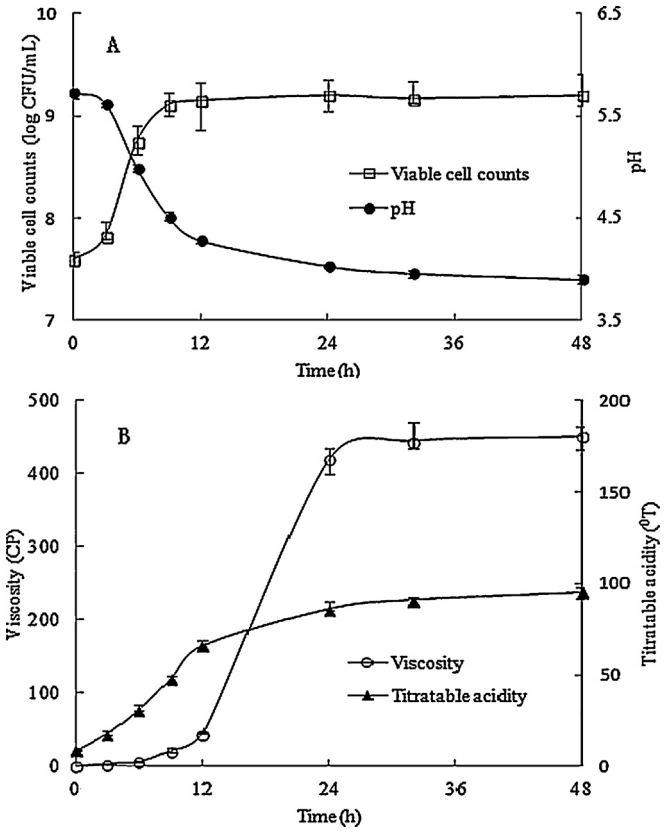

Fig. 1. Changes in: (A) viable cell counts and pH and (B) titratable acidity and viscosity during tomato-juice–sucrose medium fermentation by *L. mesenteroides* BD1710.

pH value in our study. It was interesting that the maximum viable cell count of *L. mesenteroides* BD1710 in the tomato-juice–sucrose medium reached a magnitude of 10^9 cfu mL^{-1} at 9 h of cultivation, no significant increase ($P > 0.05$) in the viable cell count could be detected with the prolongation of the cultivation time from 9 to 48 h. This might be explained by the decrease in the pH of the medium or the accumulation of organic acid as well as the depletion of nutrients in the medium (Hood & Zoitola, 1988; Shah & Jelen, 1990).

The changes in the viscosity and the titratable acidity of the tomato-juice–sucrose medium during fermentation by *L. mesenteroides* BD1710 were shown in Fig. 1B. The titratable acidity of the tomato-juice–sucrose medium increased sharply during the first 12 h of fermentation, while the viscosity of the cultured broth increased rapidly from 12 to 24 h of fermentation by *L. mesenteroides* BD1710. It had been reported that the production of high-molecular-weight dextran, which might be ascribed to the high sucrose concentration, could be responsible for the high viscosity of the gelatinous culture broth (Son et al., 2008). In our study, similar results were obtained by *L. mesenteroides* BD1710 grown in the tomato-juice medium containing 15% sucrose. The viscosity of the culture broth increased from 0 CP (centipoises) at the beginning to 451 CP at the end of the fermentation, with a remarkable increase ($P < 0.05$) from 12 to 24 h, which coincided with the bacterial cell growth of the late exponential phase and the stable phase, as shown in Fig. 1A. The viscosity of the culture broth maintained stable from 24 h until the end of the fermentation, which also indicated that the synthesis of the exopolysaccharide reached a peak.

The titratable acidity of the cultured tomato-juice–sucrose medium increased sharply from 9 to 66 °T in the first 12 h of cultivation, concomitant with the decrease in the pH, which indicated that *L. mesenteroides* BD1710 could accumulate organic acid

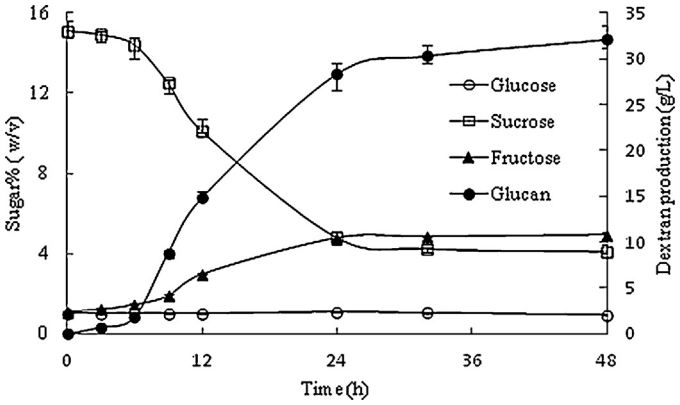

Fig. 2. Changes in the sucrose, glucose and fructose contents and the dextran yields for *L. mesenteroides* BD1710 cultivated in the tomato-juice–sucrose medium.

during metabolism. Priya et al. (2013) revealed that citric acid, malic acid and tartaric acid were the predominant acidification factors for fermented tomato juice by selected LAB strains.

3.2. Sucrose, glucose and fructose contents and dextran production

During the cultivation of L. *mesenteroides* BD1710 in the tomato-juice–sucrose medium, sucrose decreased from the initial level of 15.10 (w/v) to 4.11% (w/v) at 48 h, whereas fructose increased from the initial level of 1.11% (w/v), which might be derived from the tomato juice, to the final level of 4.93% (w/v), as shown in Fig. 2. This phenomenon was common in dextran biosynthesis by *Leuconostoc* spp., with the major portion of fructose derived from sucrose accumulated in the medium while glucose was used as substrate for dextran synthesis by dextransucrase, although some of the fructose might also be converted to other metabolites, e.g., mannitol (Hemme and Foucaud-Scheunemann, 2004). Although the glucose in the present study remained at a relatively low level of 1% (w/v), it provided sufficient carbon source for *L. mesenteroides* BD1710 to biosynthesize dextran, as observed by Moulis et al. (2006).

Dextransucrases from glycoside-hydrolase family 70 (EC.2.4.1.5) were key enzymes responsible for the dextran biosynthesis in the genus *Leuconostoc*, *Streptococcus* and *Lactobacillus* (Monsan et al., 2001). Two molecules, sucrose itself and the glucose derived from sucrose hydrolysis, acted as initiators of polymerization, and the latter was preferred when produced in sufficient amounts. Next, elongation was carried out by the transfer of a glucosyl residue derived from sucrose to the non-reducing end of the initially formed products (Moulis et al., 2006). As shown in Fig. 2, the glucose remained stable at a level of 1% (w/v) along with a significant ($P < 0.05$) decrease in the sucrose content and a sharp increase ($P < 0.05$) in the fructose content during the cultivation.

On the other hand, the dextran production by *L. mesenteroides* BD1710 in the tomato-juice–sucrose medium ranged from 0 to 32.15 g L^{-1} during the 48 h of the cultivation. The yield of dextran corresponded to approximate 60% of the glucosyl units from the metabolized sucrose. A significant increase in the dextran production occurred from 12 to 24 h (Fig. 1B), well matched with the sharp increase in the viscosity of the cultured broth, which also coincided with the bacterial cell growth of the late exponential phase and the stable phase, as shown in Fig. 1A. This indicated that the polymerization reaction with dextransucrase occurred predominantly at the late of the growth phase, and dextran was thus a secondary metabolite by the producer, as described by other researchers (Moulis et al., 2006; Vaningelgem, Zamfir, Adriany, & De Vuyst, 2004). Furthermore, the coupled increase in the viscosity of the cultured broth with the yield of dextran also indicated that the polymer had viscosity-enhancing properties, reflecting the polymer with special chemical structure, e.g., the varied degrees of branching, as reported by Scott (1972).

3.3. Comparison of dextran yields by L. mesenteroides BD1710 in the tomato-juice–sucrose medium and CDM

L. mesenteroides BD1710 performed well in dextran formation in both the tomato-juice–sucrose medium and CDM. The dextran yields in the former ranged from 0 to 32.15 g L^{-1}, whereas in the latter ranged from 0 to 29.6 g L^{-1} during the 48 h cultivation period. A solution of 5 mg mL^{-1} of the *L. mesenteroides* BD1710 dextran prepared from the 48 h cultured tomato-juice–sucrose medium contained undetectable proteins by the Bradford assay using bovine serum albumin (Sigma, St. Louis, MO, USA) as standard (Bradford, 1976). The low contents of proteins could be explained by the procedure used to prepare dextran in this study, during which the cultured broth was highly diluted, leading the proteins uneasily being precipitated by ethanol. The collected pellet was repeatedly re-dissolved in de-ionized water and precipitated by ethanol twice to remove most of the proteins, although some loss of exopolysaccharides might also occur. A slightly higher ($P > 0.05$) dextran yield in the tomato-juice–sucrose medium also indicated that it could be utilized as an alternative for the CDM.

The CDM was previously designed for *L. mesenteroides* to synthesize glucan for the advantages of: (i) reproducibility of the chemical composition of the medium; (ii) avoiding unnecessary excess of the nutrients and facilitating the adjustment of their levels; (iii) meeting the experimental determination of nutritional requirements of various strains; and (iv) supporting growth at a rationally high rate (Foucaud et al., 1997). However, whether the synthetic chemicals in the CDM were associated with potential toxicological risks had been of great concern for consumers, and a naturally derived medium is desirable in the food industry.

Tomato juice, composed of ca. 93.1% water, 4.89% carbohydrates, vitamins, and minerals, and a small amount of proteins and fats (Abdel-Rahman, 1982), is well recognized as one of the suitable media to produce probiotic beverages (Yoon et al., 2004). Substantial evidence indicated that tomato juice as a natural medium (King et al., 2007; Tsen et al., 2008; Yoon et al., 2004), as growth-stimulating factor added to other media (Babu, Mital, & Garg, 1992; Priya & Munishamanna, 2013) or itself fortified with certain nutrients (Koh et al., 2010; Priya et al., 2013), had been proven to be suitable for the cultivation of a wide spectra of LAB (e.g., *L. acidophilus*, *L. plantarum*, *L. casei*, *L. delbrueckii* and *Bifidobacterium* spp.).

3.4. Monosaccharide composition

The monosaccharide residues most frequently occurring in the exopolysaccharides synthesized by lactic acid bacteria were glucose and galactose; the less frequent were rhamnose, fructose, mannose, galactosamine, etc. Before the analysis of the monosaccharide composition of the *L. mesenteroides* BD1710 exopolysaccharide was determined by HPAEC-PAD, a solution of 5 mg mL^{-1} of the exopolysaccharide was firstly detected by the HPLC method listed in Section 2.6 to check the residual sucrose, fructose and glucose. None of the sugars could be detected, indicating the purity of the sample suitable for undertaking analysis of the monosaccharide composition. The undetectably low levels of the free mono- and disaccharide could be ascribed to the exopolysaccharide preparation procedure used, during which most of the free sugars, highly diluted and of small molecular weights, were uneasily precipitated by ethanol and remained in the supernatant.

As shown in Fig. 3, the retention time of the monosaccharide from the digested sample was 10.450 min, which was accurately

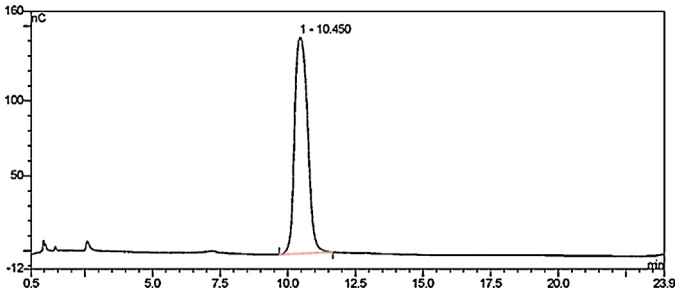

Fig. 3. Monosaccharide composition of the polysaccharide synthesized by *L. mesenteroides* BD1710 in the tomato-juice–sucrose medium.

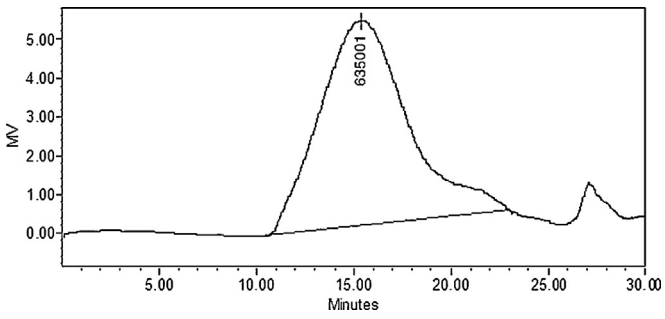

Fig. 4. HPSEC profile of dextran produced by *L. mesenteroides* BD1710 in the tomato-juice–sucrose medium.

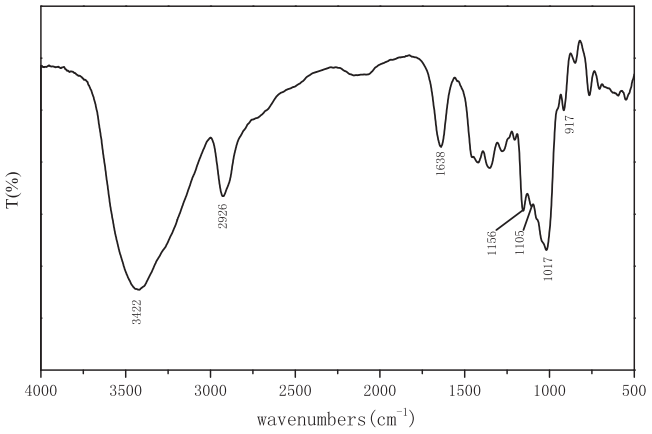

Fig. 5. FTIR spectra for dextran synthesized by *L. mesenteroides* BD1710 in the tomato-juice–sucrose medium.

matched with that of glucose, indicating that glucose was the sole monosaccharide in the polysaccharide synthesized by *L. mesenteroides* BD1710 in the tomato-juice–sucrose medium. A great number of strains in the genus *Leconostoc*, especially those belonging to *L. mesenteroides*, had been reported with the capability to synthesize glucan in various media. However, glucans differ in the type of glycosidic linkages, degree and type of branching, molecular mass, and conformation of polymers (Monchois, Willemot, & Monsan, 1999). For example, the glucans from *L. mesenteroides* NRRL B-1229 and B-512F were dextran with a large amount of α-(1 → 6) glycosidic linkages (Monchois, Remaud-Simeon, Monsan, & Willemot, 1998; Monchois, Remaud-Simeon, Russell, Monsan, & Willemot, 1997), whereas the glucan synthesized by *L. mesenteroides* NRRL B-1355 was alternan with alternating α-(1 → 6) and α-(1 → 3) glycosidic linkages (Arguello-Morales et al., 2000). Therefore, the *L. mesenteroides* BD1710 glucan was further analysed by nuclear magnetic resonance (NMR) to clarify the type of glycosidic linkages and the branching degree.

3.5. Molecular-weight distribution

Microbial exopolysaccharides vary in molecular weight (*MW*) distribution, monosaccharide composition and polymer conformation (degree of branching as well as type of glycosidic linkages), which endue the polymers with diverse structures and biological activities (He, Niu, Li, Xu, & Lu, 2012). The key enzymes responsible for exopolysaccharide synthesis with dextran as the predominant component, among the members of the *Leuconostoc* species, are glucansucrases, which can efficiently convert sucrose into dextran (Jeanes et al., 1954). Son et al. (2008) revealed that the average molecular weight of dextran was dependent upon the sucrose concentration and the nutrients in the medium. The higher the sucrose concentration used in the culture broth, the higher the molecular weight was of the dextran produced. Similar results were obtained in our study. By HPSEC, the peak molecular weight of dextran synthesized by *L. mesenteroides* BD1710 in the tomato-juice–sucrose medium was determined to be 6.35×10^5 Da, with an estimated average molecular exceeding 2×10^6 Da (Fig. 4), which might reasonably explain the high viscosity of the cultured broth, as shown in Fig. 1B. This was in agreement with the results reported by Hassler and Doherty (1990).

3.6. FTIR spectroscopy analysis

The type of the glycosidic linkages and the functional groups of dextran synthesized by *L. mesenteroides* BD1710 were characterized by FTIR spectroscopic analysis. As shown in Fig. 5, the band at 1017 cm^{-1} reflected the considerable chain flexibility present in dextran near the α-(1 → 6) linkages (Shingel, 2002). The peak at 917 cm^{-1} provided evidence for the α-glycosidic bond, and the band at 1156 cm^{-1} was due to the vibrations of the C—O—C of the glycosidic bridge. The broad peak at 1105 cm^{-1} was relevant to the vibration of the C—O bond at the C-4 position of a glucose residue (Shingel, 2002). The hydroxyl and C—H stretching vibrations of the polysaccharide caused the band in the regions of 3422 and 2926 cm^{-1}, respectively (Capek et al., 2011; Liu et al., 2007). The band in the region of 1638 cm^{-1} was due to bound water (Frank, 1971). The FTIR spectra of the dextran synthesized by *L. mesenteroides* BD1710 was very similar to those of *L. mesenteroides* NRRL B-512F and FT045B (Palmuti Braga Vettori, Martins Franchetti, & Contiero, 2012), indicating a similar structure. The α-(1 → 6) linkages were further confirmed by ^{13}C and ^{1}H NMR analysis.

3.7. ^{1}H and ^{13}C NMR spectroscopy analysis

Compelling evidence for the main structural features of dextran from BD1710 were provided by the ^{1}H and ^{13}C NMR spectra shown in Fig. 6. The low-intensity peak at 5.25 ppm (Fig. 6A) corresponded to the typical characteristics of α-(1 → 3)-linked D-glucosyl residues (Naessens et al., 2005; Seymour, Knapp, Chen, Jeanes, & Bishop, 1979). The peak at 4.91 ppm was the typical, characteristic α-(1 → 6) chain-extending anomeric signal for dextran (Chakraborty, Mondal, Pramanik, Rout, & Islam, 2004; Kim et al., 2000), which was in good agreement with the FTIR absorption peak at 917 cm^{-1} (Fig. 5). The percentage of α-(1 → 3)-linked D-glucosyl and α-(1 → 6)-linked D-glucosyl linkages was determined by integration of the signals at 5.25 and 4.91 ppm (Table 2).

The anomeric carbon signal at 98.03 ppm (Fig. 6B) confirmed that the sugar residues were α-glycosidically linked (Chakraborty et al., 2004). The peaks at 71.69, 73.92, 69.71 and 70.37 ppm corresponded to C-2, C-3, C-4 and C-5, respectively (Kim et al., 2000; Seymour, Knapp, et al., 1979). The downfield shift of the C-6 carbon signal for the glucose unit at 65.54 ppm clearly indicated that the

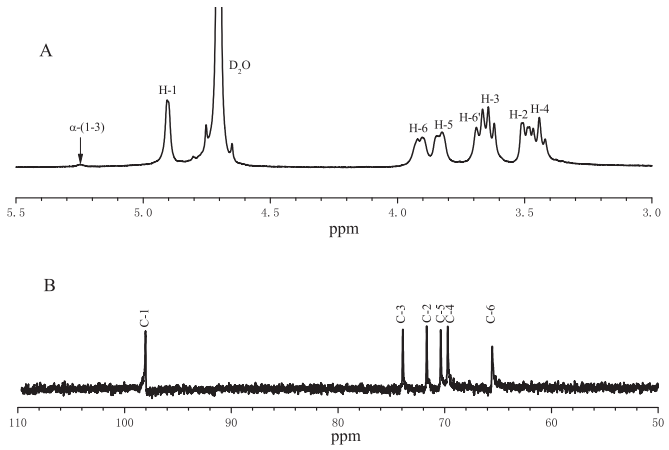

Fig. 6. (A) ^{1}H and (B) ^{13}C NMR (400 MHz, D_2O) spectra for dextran synthesized by *L. mesenteroides* BD1710 in tomato-juice–sucrose medium.

Table 1
Assignments of individual signals in ^{1}H and ^{13}C NMR spectra of the dextran from B-512F (reported) and BD1710.

	B-512F (reported values)		BD1710 (experimental)	
	^{13}C (ppm)	^{1}H (ppm)	^{13}C (ppm)	^{1}H (ppm)
C1-H	99.3	4.91	98.03	4.91
C2-H	72.9	3.50	71.69	3.51
C3-H	74.8	3.65	73.92	3.64
C4-H	71.2	3.45	69.71	3.44
C5-H	71.8	3.85	70.37	3.83
C6-H	67.3	3.93	65.54	3.90
C6′-H	67.3	3.70	65.54	3.69

Table 2
Profiles found in dextran synthesized by various *Leuconostoc* spp. strains.

Strains	Solubility	Percent of linkages (%)				
		α-1,6	α-1,3	α-1,3 Br[a]	α-1,2 Br	α-1,4 Br
L.m. BD1710[b]	Soluble	94		6		
L.m. B-512F[c]	Soluble	95		5		
L.m. B742[d]	Soluble	50		50		
L.m. B742[d]	Less soluble	87				13
L.m. B1299[d]	Soluble	65			35	
L.m. B1299[d]	Less soluble	66		7	27	
L.m. B1355[d]	Soluble	54	35	11		
L.m. B1355[d]	Less soluble	95		5		
L.m. FT045B[d]	Soluble	97.9		2.1		
L.c. B110-1-2[e,f]	Soluble	93	6			1

Note: abbreviations listed in the table.
[a] Br = branch linkage.
[b] L.m. = *Leuconostoc mesenteroides*.
[c] Data cited from Van Cleve et al. (1956).
[e] L.c. = *Leuconostoc citreum*.
[d] Data cited from Palmuti Braga Vettori et al. (2012).
[f] Data cited from Gil et al. (2008).

two glucose units in the polymer chain backbone were linked via α-(1 → 6) linkages.

The assignments for the individual signals of the dextran from B-512F (reported) (Maina, Tenkanen, Maaheimo, Juvonen, & Virkki, 2008; Palmuti Braga Vettori et al., 2012) and BD1710 in D_2O were listed in Table 1. The proton signals of the dextran from BD1710 and those from B-512F matched well, indicating that they shared a similar structure.

The glycosidic linkages of dextran synthesized by *L. mesenteroides* BD1710 as well as several other strains belonging to the same species reported were displayed in Table 2 (Gil et al., 2008; Palmuti Braga Vettori et al., 2012; Seymour, Chen, & Bishop, 1979; Van Cleve et al., 1956). When solubilized in deionized water at a concentration of 0.1%, the BD1710 dextran formed a clear, stable solution, which was important for the potential application of the polymer as a viscosity enhancer or stabilizer in food industry, for most of such food additives utilized at the level around 0.1%, e.g., the gellan. When at higher concentrations, e.g., of 0.5%, the BD1710 dextran formed a glutinous, translucent solution. Similar solubility was observed for **B-512F** dextran.

4. Conclusion

In summary, the dextran synthesized by *L. mesenteroides* BD1710 in the tomato-juice–sucrose medium had a linear backbone made of consecutive α-(1 → 6)-linked D-glucopyranosyl units and approximately 6% α-(1 → 3) branches. The BD1710 dextran could be easily solubilized in deionized water at 0.1% and at higher concentrations, e.g., 0.5% formed a glutinous, translucent solution. In addition, the tomato-juice–sucrose medium was proven to be suitable for *L. mesenteroides* BD1710 to synthesize dextran. Further study is necessary to compare the composition of the dextran synthesized by *L. mesenteroides* BD1710 in tomato juice with various sucrose concentrations as well as that synthesized in CDM to strengthen the possibility of the tomato-juice–sucrose medium as an alternative method to manufacture dextran on a large scale.

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