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Characterization and potential applications of high molecular weight dextran produced by *Leuconostoc mesenteroides* AA1

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ABSTRACT

Different strains of *Leuconostoc mesenteroides* were isolated from indigenous sources that were capable of producing a commercially important long chain glucose polymer known as dextran. Dextran produced by *L. mesenteroides* AA1 was characterized. The physicochemical characteristics and structural analysis of dextran revealed that this biopolymer has many potential applications in several biotechnological industries and *L. mesenteroides* AA1 can be used for commercial production of dextran on large scale. Dextran produced by this strain is a high molecular weight glucose polymer with only $\alpha(1 \rightarrow 6)$ branch linkage in the main backbone chain without any further branching with a viscosity of 2.23×10^3 cp of 5.0% dextran solution. This high molecular weight dextran has an average molecular weight ranging from 10,000 to 40,000 kDa. The key utility of dextran in different industries lies on its molecular weight and dextran produced by *L. mesenteroides* AA1 can be tailored to meet the requirement for any industry.

1. Introduction

Incredible increase in the demand of biodegradable biotechnological products including enzymes and biopolymers has diverted the researchers to search for more biological sources capable of producing them at industrial scale. Many microbial sources from different regions have been exploited for this purpose. One of the most important genera from microbial sources exploited for this purpose is the lactic acid bacteria (LAB). Exopolysaccharides produced from LAB have gain popularity among industrial sectors due to its generally regarded as safe status (GRAS) because LAB and their bio-products are used in several food industries (Welman & Maddox, 2003). *Leuconostoc mesenteroides* is an LAB and this epiphytic bacterium due to its presence in naturally fermented foods is considered GRAS (Holzapfel & Schillinger, 1992) and they are known to produce variety of food grade exopolysaccharides. Among them *L. mesenteroides* is used for the production of a biodegradable glucose polymer known as dextran that has several targeted industrial applications from food, cosmetics, pharmaceutical to oil drilling industries (Kim & Day, 1994; Leathere, Hayman, & Cote, 1995; Shamala & Prasad, 1995; Sutherland, Reh, Reed, Puhler, & Stadler, 1996). Among many exopolysaccharides available from microbial sources dextran has gained worldwide recognition because *L. mesenteroides* is capable of producing water soluble and insoluble dextran with diversified properties (Dols, Remaud-Simeon, & Monsan, 1997; Dols, Willemot, Monsan, & Remaud-Simeon, 2001).

Dextran is a homopolymer of glucose and is synthesized by dextransucrase in the presence of sucrose as a substrate (Santos, Teixeira, & Rodrigues, 2000). Dextran produced by different strains differs in their glucosidic linkages, degree and type of branching, mass as well as including all the physical and chemical characteristics (Cote & Robyt, 1982; Figures & Edwards, 1981; Zahnley & Smith, 1995). Production of both dextransucrase and dextran from *L. mesenteroides* is highly effected by numerous physico-chemical factors (Kobayashi, Yokoyama, & Matsuda, 1986; Lawford, Klingerman, & Williams, 1979; Lopez & Monsan, 1980; Vedyashkina, Revin, & Gogotov, 2005) and several attempts have been made by rapid advancement in biotechnological processes to improve their production. With the rapid advances in enzymology and fermentation technology, new *L. mesenteroides* strains able to produce commercially feasible quantities of dextran are being continuously searched. More recently, two new bacterial species are reported that are capable of producing dextran other than *Leuconostoc* and *Sreptococcus* species (Patel, Kasoju, Bora, & Goyal, 2010; Shukla & Goyal, 2011). Due to biodiversity, screening of a large number of dextran producing specie is an important step in selecting a highly potent culture capable of producing different types of dextran for multipurpose use.

Keeping the significance and multiple uses of different molecular weights of dextran in vision, the goal of the current study was intended to characterize the dextran produced from

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L. mesenteroides AA1 which was previously isolated from a fermented cabbage.

2. Materials and methods

2.1. Taxonomic characterization of microorganism and cultivation conditions

Identification of the genus *Leuconostoc* was based on morphological and biochemical characteristics. The organism used in the current study was isolated and identified as described previously (Aman, Qader, Bano, Iqbal, & Azhar, 2009). *L. mesenteroides* AA1 was further identified using 16S rDNA sequence analysis and gained GenBank accession number GU216257. The culture was maintained at 4 °C on tomato juice agar slant (pH 7.2) that contained ($g\,l^{-1}$) tryptone, 10.0; yeast extract, 10.0; filtered tomato juice, 200 ml and agar, 20.0.

For the production of dextran the organism was grown in a medium containing ($g\,l^{-1}$): Sucrose, 100.0; Bacto-peptone, 5.0; yeast extract, 5.0; K_2HPO_4, 15.0; $MnSO_4{\cdot}H_2O$, 0.01; NaCl, 0.01; $MgSO_4{\cdot}7H_2O$, 0.01; $CaCl_2{\cdot}2H_2O$, 0.1. The pH of the medium was adjusted to 7.5 before sterilization at 121 °C for 15 min (Aman, Qader, Bano, Khan, & Azhar, 2009). For dextran production, the inoculum was cultivated at 25 °C for 24 h containing 10^6 cells/ml. This inoculum (10%) was transferred to the fermentation broth and was incubated at 25 °C for 18 h. After 18 h dextran was precipitated. Whereas, for dextransucrase production sucrose concentration used was 25.0 $g\,l^{-1}$ and it was incubated at 25 °C for 08 h.

2.2. Enzyme assay

Dextransucrase activity was determined by measuring the reducing sugar by Nelson Somogyi method as described earlier (Kobayashi & Matsuda, 1974). A unit of dextransucrase activity is expressed in DSU (Lopez & Monsan, 1980). One unit of enzyme activity was defined as the enzyme quantity that converts 1.0 mg of sucrose into fructose and dextran in 1.0 h at 35 °C in citrate phosphate buffer (0.1 M, pH 5.0). Total protein was measured by Lowry's method (Lowry, Rosebrough, Farr, & Randall, 1951).

2.3. Estimation of total carbohydrate content and reducing sugar

Total carbohydrate content was performed using 0.2% dextran solution by using anthrone method as described previously by Hassid and Abraham (1957). For the estimation of reducing sugar, 1.0% dextran solution was used with 3,5-dinitrosalicylic acid reagent (Miller, 1959).

2.4. Determination of viscosity, average molecular weight and dextran linkages

Dextran was precipitated and purified as described previously using pre-chilled ethanol (Farwa, Qader, Aman, & Ahmed, 2008). For viscosity measurements 5.0% dextran solution was used. Viscosity was determined at 25 °C using spindle #3 with a speed of 10 rpm on Brookfield digital viscometer (DV-II). The average molecular weight of dextran was determined gel permeation chromatography analysis using XK16/70 column packed with Sephacryl S-500HR on LKB gel filtration system. It was eluted using citrate phosphate buffer (0.1 M, pH 5.0) with a constant flow rate at 30.0 ml/h. The fractions were collected using Ultro Rac II (Model LKB 2070). For the detection of dextran linkages, ^{13}C NMR and ^{1}H NMR spectroscopic analysis of dextran was performed in D_20 at 25 °C and NMR spectra was recorded using Bruker AC400 spectrometer. All the operational conditions were maintained as described earlier (Farwa et al., 2008). The Fourier-transform infrared (FT-IR) spectrum of purified dried dextran from *L. mesenteroides* AA1 and industrial grade dextran from *L. mesenteroides* 512 (Sigma) was recorded using spectrometer Nicolet Avtar 370 DTGS Smart Omni sampler (Thermo Electron Corp.) interfaced with EZ Omnic software.

3. Results and discussion

Screening of industrially important bacterial isolates capable of producing diversified type of dextran was the main objective of the current study. Eight bacterial strains belonging to genus *Leuconostoc* were isolated and purified from mixed cultures which were obtained from different vegetable and fruit samples. The pure cultures were identified on the basis of phenotypic features and chemotaxonomic characteristics. These selected strains were grown on the sucrose-containing medium to enhance their dextran producing property. From these variants, selection was made of the best strain with respect to enzyme activity. Among these strains, dextransucrase hyper-producing strain was selected and named as *L. mesenteroides* AA1 and was used for further studies for dextran production. Dextransucrase from *L. mesenteroides* AA1 has been previously characterized (Aman, Qader, Bano, Khan, & Azhar, et al., 2009). The enzyme activities and dextran production of all isolated strains is presented in Table 1. *L. mesenteroides* AA1 not only showed the highest enzyme activity but also the highest quantity of dextran produced. Although some other strains also showed high enzyme activities but the percent conversion to dextran was less as compared to strain AA1. The dextran produced from this strain was further subjected for purification and characterization.

There are several evidences available that optimization of conditions plays an important criterion for the production of dextran. Among all the physical and chemical conditions, temperature, sucrose and acceptor concentrations have played a crucial role not only on the production but also on the molecular weight of the dextran. Sucrose is the only available substrate that induces the dextran production in *Leuconostoc* strains however, dextran producing property is constitutive in *Streptococcus* species (Chellapandian, Larios, Sanchez, & Lopez-Munguia, 1998; Ciardi, Beaman, & Wittenberger, 1997). Conditions were optimized previously for dextran production using molasses for *L. mesenteroides* and it was reported that 5.4–5.5 g % dextran was produced with 17.5 sucrose in the medium (Vedyashkina et al., 2005). *L. mesenteroides* AA1 was used and was tested for its ability to produce dextran at various physical conditions. Initially the production of dextran was detected at different time intervals for strain AA1 and it was observed that cellular growth become stable after 08 h of fermentation. It was very difficult to monitor the cellular growth and dextransucrase in the fermentation broth that contained 100.0 $g\,l^{-1}$ of sucrose. Due to this high concentration of sucrose the fermentation broth become more viscous that hindered separation of both the cell mass and the enzyme, therefore 25.0 $g\,l^{-1}$ sucrose in the medium was used for this purpose. Similar results have also been reported by Santos et al. (2000) when they used sucrose concentration of 80 and 120 g/dm^3. It is clear from the data (Table 2) that maximum enzyme production was obtained after 08 h of fermentation and no further increase was noticed up to 18 h. However, maximum dextran production increased up to 18 h of fermentation from 3.11 to 4.89 g % along with the viscosity of the dextran, indicating that after 08 h when the fermentation broth become viscous it hindered cell multiplication and enzyme production. After 08 h the remaining sucrose concentration in the medium may be converted into glucose and fructose due to the presence of dextransucrase that finally increased dextran concentration and ultimately dextran viscosity. The increase in the viscosity of dextran indicates

Table 1
Production of dextran from different strains of *Leuconostoc mesenteroides*.

Strain code	Source	Dextransucrase[a] activity (DSU/ml/h)	Dextran[b] (g)	% Conversion[c]	Dextran texture	Dextran color
AA1	Cabbage	53.5	4.89	48.9	Fine powder	White
AA12	Tomato	41.8	4.57	45.7	Fine powder	Pale white
AA16	Sapodilla	48.2	4.70	47.0	Granular	Pale white
AA18	Tomato	26.1	3.95	39.5	Granular	Pale yellow
AA19	Apple	46.3	4.42	44.2	Fine powder	White
AA20	Bitter gourd	27.0	4.68	46.8	Granular	White
AA25	Ridged gourd	39.1	4.00	40.0	Granular	Pale yellow
AA27	Peach	10.8	2.55	25.5	Granular	White

[a] Dextransucrase produced using 25.0 g l^{-1} sucrose in the medium.
[b] Dextran was produced using 100.0 g l^{-1} sucrose in the medium.
[c] Calculation is based on the conversion of sucrose into dextran.

Table 2
Effect of time course on production of cellular growth, dextransucrase and dextran by *L. mesenteroides* AA1.

Fermentation time (h)	Wet cell mass[a] (g/dl)	Dextransucrase[a] activity (DSU/ml/h)	Dextran[b] (g)	% Conversion[c]	Density[d] (gm/cm^3)	Viscosity[d] (cp)
2	0.19	11.0	Nil	–	–	–
4	0.33	19.7	0.85	8.50	nd	nd
6	0.52	30.4	1.70	17.0	nd	nd
8	0.85	53.5	3.11	31.1	1.0320	1.35×10^3
12	0.84	52.5	3.89	38.9	1.0375	1.98×10^3
18	0.84	52.9	4.89	48.9	1.0430	2.23×10^3
24	0.83	40.1	4.75	47.5	1.0410	2.18×10^3
48	0.76	20.6	4.51	45.1	1.0410	2.19×10^3

nd: not determined.
[a] Cell growth was measured as wet cell mass. Cell growth and dextransucrase produced was measured using 25.0 g l^{-1} sucrose in the medium.
[b] Dextran was produced using 100.0 g l^{-1} sucrose in the medium.
[c] Calculation is based on the conversion of sucrose into dextran.
[d] 5.0% dextran solution, at 25 °C.

Table 3
Effect of sucrose concentration on production of dextran by *L. mesenteroides* AA1.

Sucrose concentration	Dextran[a] (g)	% Conversion[b]	Density[c] (g/cm^3)	Viscosity[c] (cp)
5.0	2.11	42.2	1.0210	1.05×10^3
10.0	4.89	48.9	1.0430	2.23×10^3
15.0	5.12	34.1	1.0100	1.92×10^3
20.0	6.11	30.5	1.0100	1.88×10^3

[a] Dextran was produced using 100.0 g l^{-1} sucrose in the medium.
[b] Calculation is based on the conversion of sucrose into dextran.
[c] 5.0% dextran solution, at 25 °C.

Table 4
Effect of temperature on production of dextran by *L. mesenteroides* AA1.

Temperature (°C)	Dextran[a] (g)	% Conversion[b]	Density[c] (g/cm^3)	Viscosity[c] (cp)
15	1.98	19.8	1.0300	0.85×10^3
20	3.54	35.4	1.0350	1.68×10^3
25	4.89	48.9	1.0430	2.23×10^3
30	2.80	28.0	1.0210	0.99×10^3
35	1.22	12.2	1.0100	0.65×10^3

[a] Dextran was produced using 100.0 g l^{-1} sucrose in the medium.
[b] Calculation is based on the conversion of sucrose into dextran.
[c] 5.0% dextran solution, at 25 °C.

Table 5
Effect of calcium chloride on dextran production by *L. mesenteroides* AA1.

$CaCl_2$ concentration (%)	Dextran[a] (g)	% Conversion[b]	Density[c] (g/cm^3)	Viscosity[c] (cp)
0.0	3.82	38.2	1.0300	0.65×10^3
0.001	4.45	44.5	1.0313	1.37×10^3
0.005	4.89	48.9	1.0430	2.23×10^3
0.010	4.57	45.7	1.0319	1.40×10^3
0.015	4.53	45.3	1.0315	1.39×10^3
0.020	4.51	45.1	1.0313	0.98×10^3

[a] Dextran was produced using 100.0 g l^{-1} sucrose in the medium.
[b] Calculation is based on the conversion of sucrose into dextran.
[c] 5.0% dextran solution, at 25 °C.

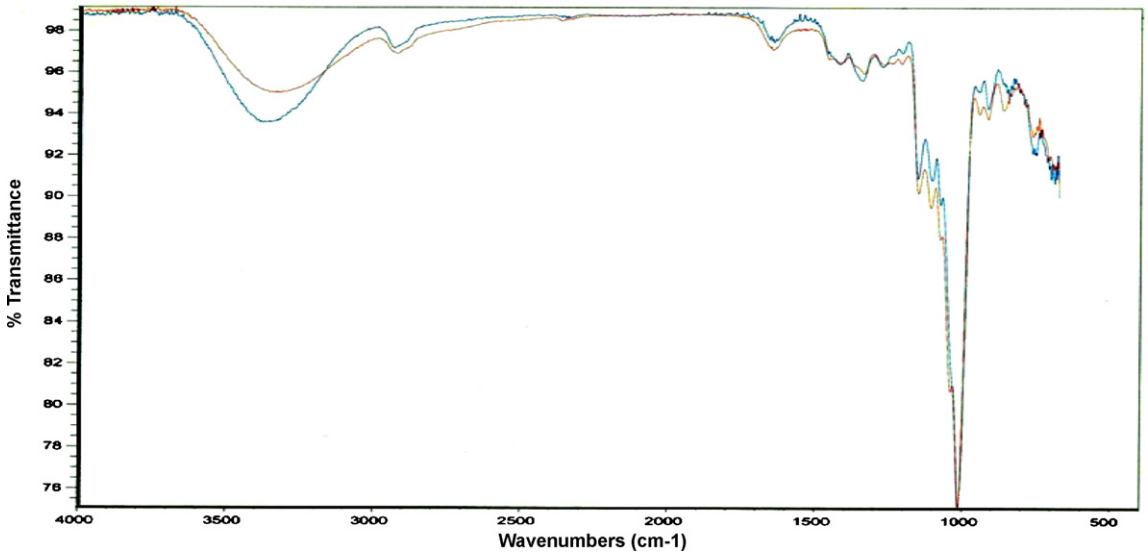

Fig. 1. FT-IR spectrum of dextran produced by *L. mesenteroides* AA1 (red) and dextran from *L. mesenteroides* 512, Sigma (blue). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

the increase in the molecular weight also which means that incubation time influence the average molecular weight of dextran up to the availability of free glucose from sucrose. Any further increase in incubation time can also lead to the formation of oligosaccharides which is not the desired by-product during the production of dextran.

In the current study, sucrose was used in the range of 5.0–20.0% and the medium containing 10.0% sucrose concentration gave the highest yield of dextran in terms of percent conversion of sucrose to dextran (Table 3). As the concentration of the substrate was raised the percent conversion rate declined and this may be due to substrate inhibition effect at higher concentrations of sucrose. The viscosity of the dextran produced was also greatly affected by the concentration of the sucrose used. The change in dextran viscosity indicates the variation of the length of dextran and after 10.0% the percent conversion of sucrose to dextran decreased with the decrease in viscosity.

In the current study production and the viscosity of the dextran from *L. mesenteroides* AA1 was also monitored at different temperatures (Table 4). A marked difference in production as well as in viscosity was observed and maximum production and percent conversion of sucrose into dextran was found at 25 °C. As the temperature was raised a drastic decline in viscosity and % conversion was also noticed indicating that at higher temperature the rate in the increase of chain length of dextran is lowered. This may be due to the effect of temperature because the suitable temperature for dextransucrase production of *L. mesenteroides* AA1 was 25 °C (Aman, Qader, Bano, Iqbal, et al., 2009).

After confirming the temperature, another factor which had a distinct impact on production of dextransucrase was varied i.e. calcium chloride. Calcium chloride is also known to stabilize the dextransucrase for up to 120 days (Qader, Aman, Bano, Syed, & Azhar, 2008). Keeping this in view effect of calcium chloride was studied for dextran production and it was noticeable that by varying the concentration of calcium chloride it not only affects enzyme activity but also the dextran production and molecular weight with reference to its viscosity. Dextran production in the medium without $CaCl_2$ is less as compared to the mediums containing $CaCl_2$ and this is because dextransucrase produce in this medium is unstable and losses its activity up to 18 h which is the required time for fermentation of dextran. $CaCl_2$ as an additive has a positive influence over dextransucrase and dextran production by *L. mesenteroides* AA1. It was observed that incorporation of 0.005% calcium chloride in the medium produces maximum dextran quantities (Table 5).

Table 6
Characterization of dextran from *L. mesenteroides* AA1.

Characteristics of purified dextran	*L. mesenteroides* AA1	*L. mesenteroides* CMG713[c]	*L. mesenteroides* EA6[d]
Average molecular weight (kDa)	10,000–40,000	5000–20,000	5000–20,000
Density[a] (g/cm^3)	1.043	–	–
Viscosity[a] (cp)	2.23×10^3	14.58	1.28×10^3
Linkages	α (1 → 6) only	α (1 → 6)	–
Moisture content (%)	9.5	10.2	10.7
Ash content (%)	8.5	9.09	9.09
pH[b]	6.8	6.3	–
Total carbohydrate (%)	89.0	79.0	76.0
Reducing sugar (%)	0.8	1.0	1.08
Total protein (%)	1.0	1.9	–

[a] 5.0% dextran solution, at 25 °C.
[b] 1.0% dextran solution, at 25 °C.
[c] Farwa et al. (2008).
[d] Qader et al. (2007).

Purified dextran from *L. mesenteroides* AA1 was subjected to FT-IR analysis in comparison with the industrial grade dextran available from sigma (Fig. 1) and the spectrum provided the data confirming that the dextran in the current study contains all the functional groups, monomeric units and the linkage present is α-(1 → 6) glycosidic bond i.e. peaks near the regions 906 cm^{-1} and 1020 cm^{-1} (Shingle, 2002). The linkage was also further confirmed by ^{13}C NMR and ^{1}H NMR spectroscopic analysis (data not shown).

The physico-chemical features of dextran produced by *L. mesenteroides* AA1 is shown in Table 6. Tremendous diversity of microbial species has contributed enormously in the industrial revolution and biopolymers from biological resources have proved to be the major asset of several biotechnological industries. Among several bio-products available in the market, one of the major contributions in various industrial sectors is of biopolymers and dextran is one of the few biopolymers that have multiple uses. Comparison of dextran characteristics produced by *L. mesenteroides* AA1 with other previously reported dextran revealed that due to its high molecular weight & viscosity characteristics, this dextran can be used for several industrial purposes after hydrolyzing it into different low molecular weight fractions of different grades.

Commercially *L. mesenteroides* B-512F is used for the production of dextran and the percent conversion of sucrose to dextran by this strain is reported to be 41.0 (Qader, Iqbal, Rizvi, & Zuberi, 2001) as compared to *L. mesenteroides* AA1 which is 48.9%. It is evident that yield of dextran and it molecular weight from *L. mesenteroides* AA1 is higher is as compared to previously reported strains (Farwa et al., 2008; Qader, Shireen, Aman, Iqbal, & Azhar, 2007). The key points for production of dextran in industry are it molecular weight and dextran yield and keeping these point in view *L. mesenteroides* AA1 is a potential candidate for dextran production at industrial scale. In industrial grade form, this high molecular weight dextran from *L. mesenteroides* AA1 can be used in oil drilling industries as dextran-aldehyde complex or as protective colloid during mud drilling operations. As a colloidal preparation it can also be conjugated with iron and other chemicals to prepare different derivatives that can be further used for various pharmaceutical purposes.

4. Conclusions

The economic importance of lactic acid bacteria (LAB) drives from the fact that they can be exploited by humans in a number of beneficial ways. Recent advances in microbial research have created a great impact on various industrial processes and have improved yield of many biotechnological products with nutritional values using different species of LAB. Currently, they are used for the production of biopolymers, enzymes, wines and many other products of commercial importance. Dextran has broad applications in various biotechnology industries and they have made a remarkable impact in the world of glycotechnology. The physical and chemical properties of dextran vary depending upon the type of strain used in most of the cases and the dextran produced by *L. mesenteroides* AA1 is water soluble and is tasteless solid. All the commercial dextran producing industries are producing dextran of different grade and molecular weights. The dextran produced by *L. mesenteroides* AA1 has remarkable potential to be used for several industrial purposes and this strain can be used for the elaboration of the dextransucrase on a commercial scale as well. The dextran from this strain can be hydrolyzed into several molecular weight fractions and can be conjugated with different compounds depending upon its utility in the biochemical research laboratories or industries. Furthermore, multiple grades can be manufactured using *L. mesenteroides* AA1 by varying the fermentation conditions.

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