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Production and characterization of dextran from *Leuconostoc mesenteroides* NRRL B-512(f) fermentation

Faqiha Munir[1,2,3], Yasar Saleem*[1], Nabiha Munir[1,3], Muhammad Shaheen Iqbal[1,3], Sania Sattar[3]

[1] Food and Biotechnology Research Center (FBRC), Pakistan Council of Scientific and Industrial Research (PCSIR) Labs Complex, Lahore, Pakistan.
[2] Institute of Molecular Biology and Biotechnology, University of Veterinary and Animal Sciences (UVAS), Lahore Pakistan
[3] Institute of Molecular Biology and Biotechnology, University of Lahore (UOL), Lahore Pakistan

***Corresponding Author**
Dr. Yasar Saleem (ysaleem73@hotmail.com)
Food and Biotechnology Research Center (FBRC), Pakistan Council of Scientific and Industrial Research (PCSIR) Labs Complex, Lahore, Pakistan

Abstract

Dextran is a complex, branched glucose polymer carbohydrate with variable glycoside linkages and important with respect to food, clinical and medical point of view. The aim of the current study was to produce dextran from *L. mesenteroides* NRRL B-512(f) fermentation by altering carbon sources in growth media, and comparatively analyze characteristics of resulted dextran samples and its related enzyme i.e. dextransucrase. The desired strain was received from Karachi University, Karachi Pakistan. Dextran samples were fermented by *L. mesenteroides* NRRL B-512(f) using sucrose, dextrose, and fructose as carbon sources in fermentation jars under standard growth conditions. Samples were extracted and purified by treating with chilled ethanol repeatedly and characterized by analyzing physical properties, production yields, gelling properties, melting points, and molecular weights. Molecular weights of extracted dextran samples were determined on FPLC apparatus manually. Dextransucrase (DS) is involved in dextran production in the presence of a rich carbon source. Dextransucrase was extracted and purified by treating supernatants of samples with 25% pre-chilled PEG400 leading to overnight incubation and then centrifugation. The purified protein samples were collected in the form of the palate with gradual monitoring of dextransucrase activity (DSU). Subsequently, its molecular weight was also determined using SDS-PAGE. Dextran can be produced by varying the substrates in fermenting media. But its properties altered by altering the substrate. This alteration may be effective in the context of its applications. As a conclusion to this study, sucrose is the most valuable substrate for fermentation as is gives maximum yield.

Key Words: Dextran, Dextransucrase (DS), Sucrose, Fructose, Dextrose, Optical Density (OD), Fast Protein Liquid Chromatography (FPLC)

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Introduction

Dextran is an extracellular bacterial homo-polysaccharide, a complex, branched glucan (glucose polymer) composed of various glucose chains of varying lengths from 3 to 2000 kDa (1). Dextran is uninhibitedly dissolvable in polar solvents like water, methyl sulphoxide, formamide, ethylene glycol, glycerol, and so forth. Some dextran divisions may embrace a specific level of crystallinity and may just be brought into the arrangement by solid warming. Dextran delivered by Leuconostoc *mesenteroides* NRRL B-512(F) comprises of an α-1,6-glycosidic linkage with side chains connected to the 3-positions (α-1,3 linkages) of the backbone in glucose units (2).

Auxiliary and biochemical portrayal of dextran differ by changing its aging conditions like brooding temperature and term, carbon, mineral, and amino corrosive sources. It is delivered at the mechanical dimension by the aging of sucrose-rich media. Raymond and his examination specialists (1993) upgraded the aging conditions for the greatest creation of dextran (3). It has been accounted for before that atomic weight and yield of dextran creation relies upon the procedure factors, for example, temperature, sucrose, and the acceptor focus. It was additionally referenced that medium containing nitrogen source enhanced with various salts expanded its creation (4).

Dextran has heaps of business applications as a medication, an adjuvant, an emulsifier, a transporter, and stabilizer.

Cross-connected dextran is known as Sephadex, which is generally utilized for the detachment and refinement of protein. In sustenance, industry dextran is as of now utilized as jam and dessert thickener. It averts crystallization of sugar, improves dampness maintenance, and keeps up flavor and presence of different sustenance things (5).

Dextransucrase created by the L. *mesenteroides* strains polymerizes the glucosyl part of sucrose into dextran (6). These are extracellular proteins with the double working of beginning polymerization and furthermore, present the numerous sorts of the branch focuses without including anyone catalyst (7). This gathering of glucansucrases is expansive particles of normal mass 160 000 Da (8). Sucrose molecules are hydrolyzed as vitality hotspot for biosynthesis reason. Levans (fructans) are shaped by a comparable procedure in which the fructose buildups are polymerized and the glucose is acclimatized. The protein catalyzing their

arrangement is levansucrase. Without sucrose, a few microscopic organisms are fit for the creation of dextran and fructan and different polysaccharides. The significant contrast from the union of bacterial heteropolysaccharides (and different homopolysaccharides) is the absence of inclusion of sugar nucleotides and the extracellular idea of the biosynthetic procedure. (6).

Objective of the study was a comparative characterization of dextran samples by altering fermentation protocols with sucrose, fructose and dextrose (glucose) as carbon sources and then characterize the product dextran by Physical characteristics (color, texture, solubility, etc.), Enzyme activity, Production yield, Gelling property, Melting point and Molecular weight.

Materials & Methods

Strain Revival:

A purified *strain of L. mesenteroides* NRRL B-152(f) was maintained on the slant of on 10% sucrose medium and revive to fresh culture by incubating at 26ºC (5).

Confirmation

Confirmation of desired strain was done by selective medium growth (9), biochemical and antibiotic sensitivity tests (10). The 10ml inoculum was prepared with 10% sucrose broth medium and incubated at 30oC for 24 hours then transferred to 90 ml sterile broth and incubates for next 24 hours at 30oC.

Differential fermentation

Differential fermentation of *L. mesenteroides* was carried out by using 10% dextrose, fructose, and sucrose respectively in each 1 litter fermenter as a carbon source with nutrient broth as a medium for dextran production under slandered conditions.

Dextran Extraction & Purification

Each cultured broth was centrifuged at 5,000 rpm for 30 minutes and the supernatant was treated with chilled ethanol in equal volume to precipitate extracellular dextran. For purification, the extracted dextran samples were dissolved in chilled dH_2O to make a past then treated with chilled ethanol in equal volume and centrifuged at5.000 rpm for 10 minutes to collect dextran in pallet form (11). Following method was repeated thrice then resulted purified dextran samples were dried in in dryer oven and used for characterization.

General Characteristics of Dextran

General physiological characters i.e. color, texture, smell, etc., comparative percentage yield by total dry weight (g/L) extracted, relative melting points by melting point apparatus, gelling capacity and molecular weights of dextran samples were comparatively analyzed. Dextran samples were dissolved in dH_2O with 0.5, 1, 1.5 and 2 mg/ml concentrations and freeze at 4ºC to estimate their increasing thawing time against dH_2O.

Molecular weight of dextran

The molecular weight of dextran samples was calculated by FPLC apparatus following the size exclusion chromatography method against the standard blue dextran having molecular weight 200,000 Da with 1ml/min flow rate and 3ml friction size. 5ml column of Sephadex G_{200} was used for this chromatography. Citrate-phosphate buffer with pH 5.0 is used as mobile phase.

Dextransucrase Extraction & Purification

Dextransucrase is an enzyme responsible for the production of dextran was extracted from the supernatant of fermented broth of samples after 5000 rpm centrifugation and treated with pre-chilled 25% PEG-400 respectively followed by 12000rpm centrifugation for 15 minutes and the enzyme was settled down in form of the pallet.

Dextransucrase Activity & Molecular weight

The protocol described by Shukla *et al.* 2010 was followed to measure enzyme activity of dextransucrase regarding each sample in "DSU" unit defined by Miller (13). Incubated at 30º C for 1 hour and ODs were taken at 500nm Slandered SDS-PAGE protocol was performed to determine molecular weights of sample enzymes (14).

Results

Re-cultured growth results of *L. mesenteroides* on sucrose-rich and selective media showed white, shiny, convex colonial growth with lactic acid fermentation and gas production.

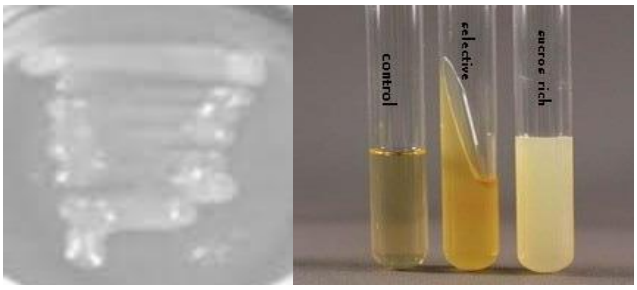

Figure 1: (a) Re-cultured growth of Leuconostoc *mesenteroides* on sucrose rich agar. **(b)** *L .mesenteroides* growth on sucrose rich and special medium along with control broth.

Biochemical tests results include, mannitol +ve and vancomycin sensitivity with 23mm inhibition zone confirmed the presence of *Leuconostoc mesenteroides*.

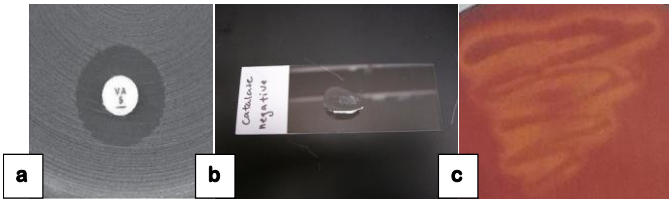

Figure 2: (a). In the Vancomycin sensitivity test, *L. mesenteroides* showed 23mm inhibition zone **(b).** *L. mesenteroides* showed the catalase –ve results as no gas formed **(c).** The color change from red to orange showed +ve mannitol results by *L. mesenteroides*

Purified dextran samples were subjected to physical characterization. All dextran samples showed no change in color, smell, texture, and solubility as shown in Table No. 1. While, change in fermentation media affect the percentage yielding, gelling property, melting point and molecular weights of dextran samples. Enzyme activity and molecular weight of dextransucrase extracted and purified from differential fermented broth showed observable variation due to change in carbon source.

Table No. 1: Comparative physical characters of dextran from different substrates

Sr. No.	Characteristics	Ds	Dd	Df
1	**Color**	White	White	White
2	**Smell**	No	No	No
3	**Texture**	Powder	Powder	Powder
4	**solubility (H_2O)**	5%	5%	2%
5	**solubility (ethanol)**	insoluble	insoluble	Insoluble

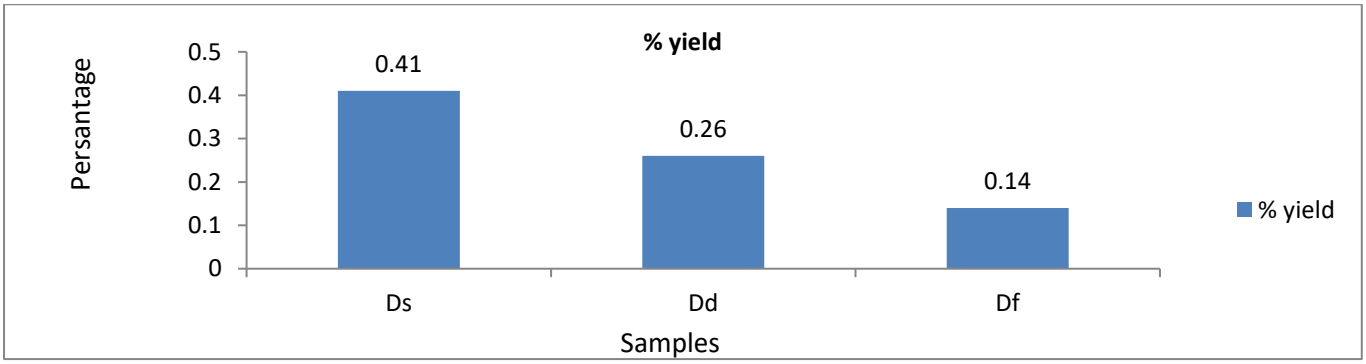

Figure 3: Comparative yield (percentage) of dextran from different substrates

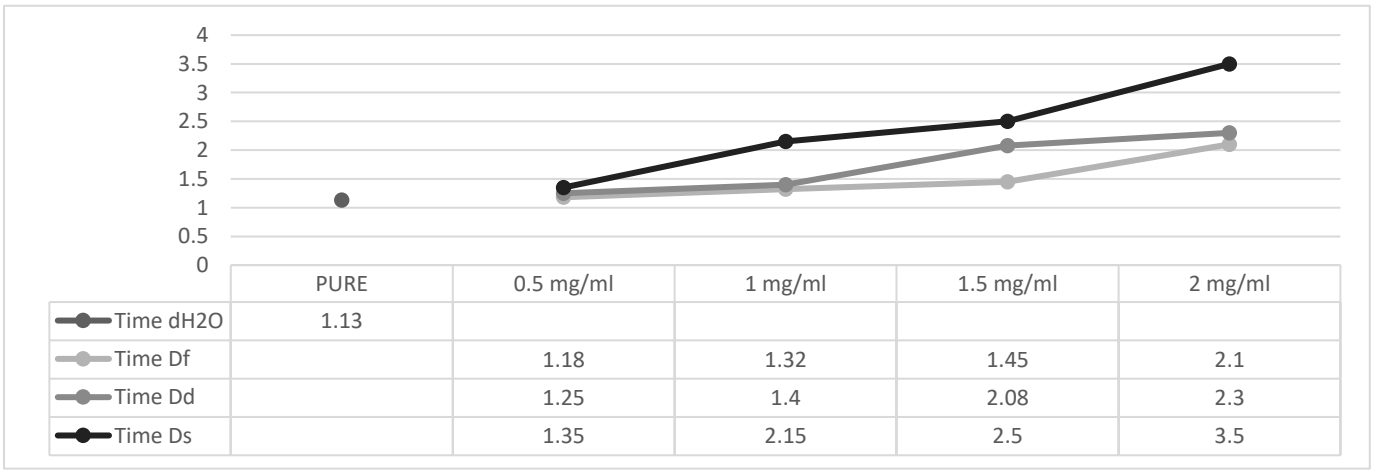

Figure 4: Gelling property of dextran from variable substrates

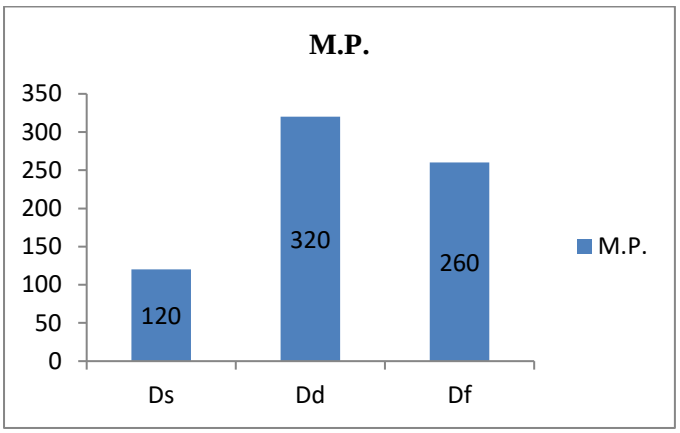

Figure 5: Comparative melting point of dextran samples

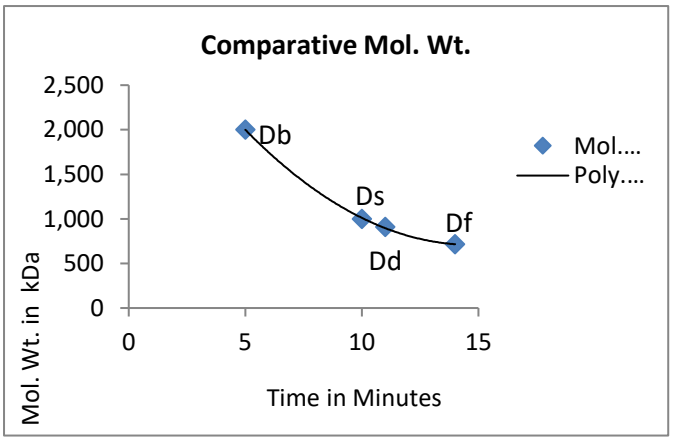

Figure 6: Comparative mol. weight of dextran samples by size exclusion chromatography on FPLC.

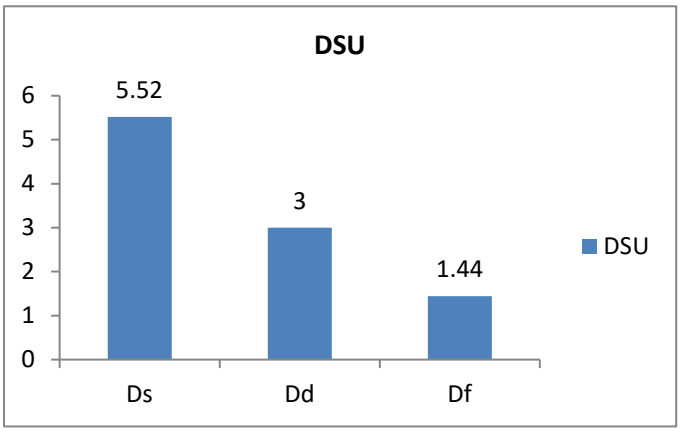

Figure 7: 1 hour incubation at 30°C, OD at 500nm in 0.1M sodium acetate buffer pH 5.0

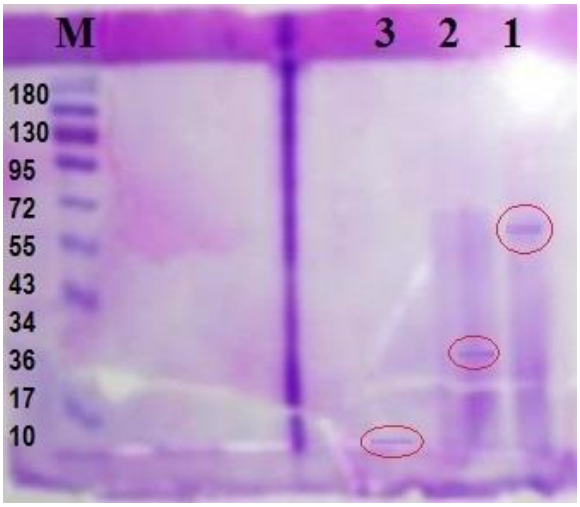

Figure 8: SDS-PAGE analysis, results show that dextransucrase fermented in sucrose medium had higher molecular weight than dextransucrase fermented in dextrose and fructose. M= molecular weight marker, lane 1=DSS (dextransucrase from sucrose fermenter), lane 2= DSF (dextransucrase from fructose fermenter), lane 3 = DSD dextransucrase from dextrose fermenter)

Discussion

In 2008 Nasab *et al.* produced dextran by *Leuconostoc mesenteroides* NRRL B512 (f) using date extract v/s sucrose as carbon sources. Low molecular weight dextran samples attained by date extract then sucrose sources compared to blue dextran of molecular weight 2,000 kDa (16). While in the present study, sucrose, dextrose, and fructose were used as a carbon source in medium but the same standard, dextran blue was used to analyze molecular weights of samples. Sucrose sample showed the highest molecular weight among all.

Dols *et al.* 1997 studied the specific growth rate under aerobic conditions by using glucose and fructose supplements and found that glucose gave a batter yield in anaerobiosis. According to them, both sugars are phosphorylated and catabolized. They also found that in sucrose grown culture, sucrose converted into dextran and fructose and inhibit the growth of *L. mesenteroides* (17). On the current study, dextrose was used instead of glucose against fructose and sucrose for comparative analysis in only aerobic condition and yield of dextrose sample was least as compared to the other two. Maximum yield was attained by the sucrose sample.

Sarwat *et al.* in 2008 analyzed the effect of variable parameters including pH, temperature, %age sucrose content and incubation time and concluded that maximum production was attained with 15% sucrose after 20 hours at 30°C in 7pH (11). However, in recent work done, the comparison was done between different carbon sources and the best results were received from the sucrose sample.

Dols *et al.* in 1998 produced DS in glucose and fructose instead of sucrose and analyzed by SDS-PAGE. Dextran produced from these enzymes had variable glycosidic linkages (15). Instead of this, in ongoing work done, enzyme activity was analyzed by calculating DSU

(dextransucrase unit) in media supernatants by taking ODs before and after incubation.

Purama *et al.* in 2009 studied the dextran produced by Leuconostoc *mesenteroides* NRRL B640 and found that dextran has a novel food gelling and thickening properties (18). It was also observed in the existing study that dextran had some gelling property and increased the thawing time of solutions or medium in which they added.

Conclusion

Findings of this study concluded that dextran, as a biopolymer, its properties can be very by altering the fermenting conditions. Sucrose is used as a basic carbohydrate source in the medium for production. However, it is seen that dextran produced by using substrate other than sucrose but the yield and characteristics vary according to each supplement. Sucrose fermentation gives more effective results as compared to others. It is observed that yield, gelling capacity and molecular weights decreased when fermented with dextrose and fructose instead of sucrose. While melting points were increased. Besides all these, dextran showed the same physical characters include color, texture, and solubility.

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