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Production of Dextran by *Leuconostoc mesenteroides* Isolated from Home Made Fermented Foods

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ABSTRACT

Dextran gum has important medicinal applications. The study was aimed to produce the dextran from *Leuconostoc mesenteroides* isolated from home made fermented foods. Two strains of *Leuconostoc mesenteroides* (LM-1 and LM-2) were isolated from chilli pickle and sauerkraut by enrichment culture technique. The isolates were subjected to dextran gum production using standard sucrose medium and modified medium made with sugar cane juice and & liquid jaggery. Dextran gum produced by each isolate with different medium combinations was extracted and purified and subjected to conformity tests. The dextran produced by isolates was found to be viscous and soluble in water. The isolate LM1 showed highest yield of 3.50 g / 100ml with sugarcane juice medium. Sugarcane juice can be used as a source of sucrose which is economically affordable. However, more studies should be carried out to explore the process of dextran gum production

***Key words**: Leuconostoc, Fermented foods, Liquid jaggery, Cane juice, Dextran gum*

Introduction

Dextrans, a type of homopolysaccharides, are naturally occurring glucose polymers that have α(1,6)-linkages as the main linear chain structure. These chains can also have different kinds of branched linkages, including α(1,2), α(1,3), and α(1,4), depending on the action of an extracellular enzyme called dextransucrase. Dextransucraseis synthesized by various organisms, such as *Leuconostoc*, *Lactobacillus, Streptococcus*, and *Weisella* in the presence of sucrose as a substrate (Leathers, 2002; Neubauer, *et al.,* 2003; Naessens *et al.,* 2005 and Goulas *et al.*,2004).

Dextran, a polysaccharide, has significant medical and food industry applications, including its use as a plasma substitute, as well as in the production of fine chemicals and texture improvement in milk-based drinks, yogurts, and ice creams (Neubauer *et al.,* 2003). Dextran has proven to be a versatile compound with a wide range of applications in the food, pharmaceutical, and chemical industries (Naessens *et al.*, 2005). Its diverse properties make it a valuable ingredient as an adjuvant, emulsifier, carrier, and stabilizer (Goulas *et al.,* 2004). One of the well-known derivatives of dextran is cross-linked dextran, commonly known as Sephadex, which is extensively utilized in protein purification and separation.

In the food industry, dextran finds significant application as a thickener for jams and ice creams. Its unique ability to prevent sugar crystallization, retain moisture, and preserve the flavor and appearance of various food items make it a popular choice among food manufacturers (Qader *et al.,* 2007, Seymour *et al.,* 1980 and Purama *et al.,* 2005). Thus, it is evident that dextran is a multi-purpose compound with numerous beneficial properties that make it an indispensable ingredient in several in-

dustrial applications

The *Leuconostoc* genus pertains to the 'sensustricto' cluster of lactic acid bacteria (LAB). In plant materials, roughly 12% of LAB isolates are *Leuconostoc* species. These microorganisms can be obtained from the exterior of a broad range of fruits and vegetables, as well as from refrigerated meats and dairy products. The *Leuconostoc* group is of particular commercial importance owing to its capacity to synthesize fragrant compounds, valuable polysaccharides, and malolactic fermentation. Like other LAB clusters, *Leuconostocs* require intricate media due to their multifaceted needs for amino acids, peptides, carbohydrates, vitamins, and metallic (Sarwat *et al.,* 2008).

Dextran is reported to be produced from sucrose by strains of *Leuconostoc sp*. However, it can also be produced by *Streptococcus and Acetobacter* in sucrose-containing medium. Growing cells secrete an enzyme called dextransucrase into the medium, which converts sucrose into dextran (Behravan, J., *et al.,* 2003).

Material and Methods (Naessens *et al.,* 2005 and Qader *et al.,* 2007)

Collection of Samples: Two different samples were collected 0n 10-g amount each for the study in triplicates.The first sample was homemade chilli pickle and the second was vegetable sauerkraut prepared in the laboratory.

Isolation of *Leuconostoc mesenteroides*

To isolate *Leuconostoc mesenteroides*, one milliliter sample from each of the fermented sauerkraut and homemade chili pickle was separately inoculated into 100 mL sucrose broth. The flasks were kept for incubation at room temperature for 48 h. After 48 h, one loopful sample from each flask was streaked on *Leuconostoc* agar medium (Deshmukh, 2001). The plates were kept for incubation for 48 h at room temperature. After 48 h, plates were observed and the typical sticky, pearl-like colonies showing highly viscous slimy growth on *Leuconostoc* agar plates were selected. The typical pearl-like colonies were selected and restreaked on *Leuconostoc* agar plates. Plates were incubated for 48 h, and pure cultures of isolates were preservedin triplicateson *Leuconostoc* agar slants at 4 °C.

Study of Cultural, Morphological, and Gram Staining Properties of isolates

The suspension of the isolates was streaked on *Leuconostoc* agar and incubated at room temperature for 48 h. The colony characteristics of isolated colonies on *Leuconostoc* agar were observed, and Gram properties of isolates were studied by Gram staining method (Hucker modification) (Hucker *et al.,* 1930). Motility was studied by the hanging drop technique.

Study of Biochemical characteristics of the isolates

Biochemical characteristics of the isolates were studied with following tests

Enzymatic Characteristics: The production of various enzymes like proteases, lipases and their activities were studied by using standard methods and materials.

Fermentation of Carbohydrates: Fermentation of carbohydrates was studied for testing the ability of the organism to utilize carbohydrates as a carbon and energy source. The study was carried out by using peptone water as a basal medium containing bromothymol blue indicator and 1% solution of carbohydrates such as sucrose, glucose, fructose, maltose, and mannitol, lactose. Acid production was detected by observing the development of yellow color, and gas was not detected as bubbles in Durham's tube (Holt, 1994).

Growth Response at 15 °C and 45 °C: A loopful of suspension of each isolate was inoculated in 5 ml sterile sucrose broth tubes separately. All tubes were incubated at 15 °C and 45 °C for 48 h. After incubation, growth was checked by observing the development of turbidity.

Optimization of Sugar Source for Dextran Production using Isolates of *Leuconoctoc mesenteroides:* Dextran gum production was carried out using isolated strains of *Leuconostoc mesenteroides* as fermenting organisms and sucrose powder, sugarcane juice, and liquid jaggery as a source of sucrose. Liquid jaggery and sugarcane juice were used for dextran production.

Three different types of media were prepared. Standard sucrose broth Medium, Sugarcane juice broth Medium and Liquid Jaggery broth Medium

i) **Standard sucrose broth medium**: Sucrose 30-g+ bacto-peptone -1g + yeast extract1-g + K_2HPO4-3g, MnCl2.H_2O, 0.01-g; NaCl, 0.01-g; $CaCl_2$, 0.05-g,pH was adjusted to 7.0 [14]

ii) **Sugarcane juice broth medium**: 20 ml sugarcane juice + bacto-peptone 1g + yeast extract 1g+ K_2HPO_4, 3g $MnCl_2.H_2O$, 0.01g; NaCl, 0.01g; $CaCl_2$, 0.05g. pH was adjusted to 7.0

iii) 20 ml Liquid Jaggarery + bacto-peptone1g; yeast extract 1gm; K_2HPO_4, 3g; $MnCl_2.H_2O$, 0.01g; NaCl, 0.01g; $CaCl_2$, 0.05g. pH was adjusted to 7.0 After 48 h culture became very viscous and pH dropped from 7.5 to 5.5.

Production of Dextran

Each 10 ml of sterile sucrose broth, 10 ml of sugarcane juice medium and 10 ml sterile liquid jaggery medium were inoculated with loopful suspension of isolates, separately. All the tubes were incubated at room temperature for 48h. After incubation these 10 ml of inoculate were transferred to 200 ml respective medium broths. All the flasks were incubated at 28 ^{0}C for 48h.

Recovery of Dextran by precipitation method

The culture media after 20 h were precipitated using equal volumes of chilled ethanol, shaken vigorously, centrifuged at 10,000 rpm for 15 minutes and the supernatant was decanted. This step was repeated twice. The precipitated dextran was dried under vacuum over calcium chloride at 30 ºC. The dextran yield was calculated on dry weight basis (Sarwat *et al.*, 2008).

Purification of dextran: For removal of impurities, dextran obtained from precipitation was dissolved in distilled water. The dextran slurry was again precipitated with equal volume of chilled ethanol. This procedure of re-dissolving, precipitation and washing was repeated thrice to remove cells debris. Purified dextran was dried under vacuum over calcium chloride at 30 ºC (Sarwat *et al.*, 2008).

Confirmation of the purified product as a dextran

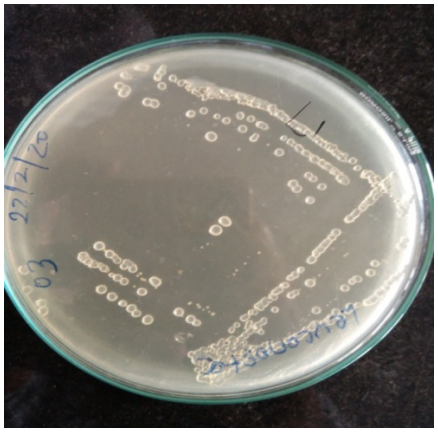
Solubility of dextran- Solubility of dextran was determined by using different chemicals for solubility in ethanol, glycerol, ethylene glycol, water, formamide, methyl sulphate, acetone, methanol and isopropanol (Behravan *et al.*, 2003).

Determination of viscosity: Viscosity was determined by viscometer.

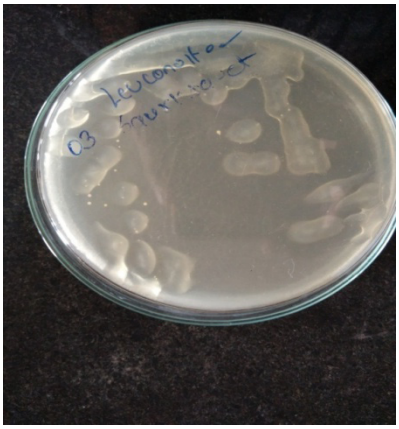
Determination of sugar content- Estimation of sugar content of dextran was determined by Benedict's method.

Results and Discussion

Isolation of ***Leuconostoc mesenteroides*****:** Two isolates designated as LM1 and LM2 were obtained from three samples each of chilli pickle and sauerkraut, respectively. From study of cultural, morphological and biochemical characteristics both the isolates were tentatively identified as a strains of *Leuconostoc mesenteroides* (Photoplates 1 & 2).



Photoplate 1. Isolate- LM1: pearl like mucoid colonies



Photoplate 2. Isolate -LM2 pearl likemucoid, large colonies

The results showed that both isolates produced more dextran gum when grown in the presence of sugarcane juice or liquid jaggery as a sugar source as compared to the standard medium with sucrose. Isolate LM1 produced the highest yield of dextran gum (3.5 g/100 mL broth) when grown in medium containing sugarcane juice, while isolate LM2 produced the highest yield (3.2g/100 ml of medium) with liquid jaggery as a sugar source (Fig. 1).

When grown in the standard medium with su-

crose powder, isolate LM1 produced 3.05g.dextran/ 100mL while isolate LM2 showed a lower yield of 1.6g /100 ml. When grown in medium containing liquid jaggery as a sugar source, isolate LM1 produced 0.35g dextran/ 100mL while isolate LM2 showed a good yield of 2.75g /100 Ml (Fig. 1).

The results of this study also show that both isolates produced dextran with similar viscosity when grown in the standard sucrose mediumor medium containing sugarcane juice. The similarity in viscosity suggests that the type of sugar source used for the growth of *Leuconostoc mesenteroides* may not significantly affect the properties of dextran produced, except for the viscosity.

Results of determination of solubility of Dextran in various solvents

The results of the solubility test show that dextran gum produced by both LM1 and LM2 strains were soluble in glycerol, ethylene glycol, formamide, water, and methyl sulphate when using sucrose, sugarcane juice broth, and liquid jaggery broth as the sugar source. However, the dextran produced from all three sugar sources by both strains were insoluble in ethanol, isopropanol, acetone, and methanol (Table 2-3).

Top of Form

The insolubility of dextran in these solvents may be attributed to the polarity of the solvent. Ethanol, isopropanol, and acetone are polar solvents and have a lower dielectric constant as compared to water, which makes them less suitable for dissolving polar compounds such as dextran.

Discussion

The results of this study suggests that the type of sugar source used for the growth of *Leuconostoc mesenteroides* significantly affects the yield of dextran gum. Isolate LM1 from chilli pickle showed a higher yield of dextran gum than isolate LM2 that from sauerkraut, regardless of the source of sucrose used. However, isolate LM2 showed a better yield with liquid jaggery as a sugar source as compared to the standard medium (Fig. 1).

The use of natural sugar sources such as sugar-

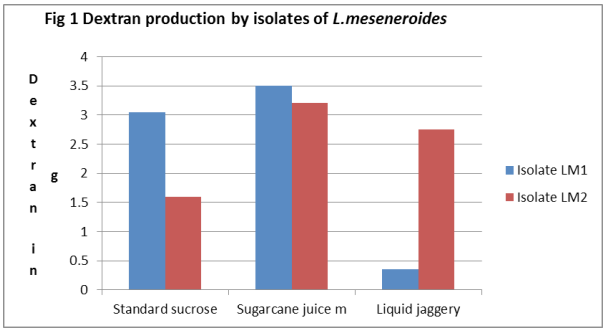


Table 1. Results of Determination of Viscosity for Dextran produced

Medium source	Viscosity-Isolate LM1	Viscosity-Isolate LM2
Standard medium	0.005-0.01Pa.s	0.005-0.01Pa.s
Sugarcane juice	0.005-0.01Pa.s	0.005-0.01Pa.s
Liquid jaggery	<0.005 Pa.s	<0.005 Pa.s

Table 2. Determination of Solubility of dextran produced by isolate LM1Top of Form

Solvent	Solubility of Dextran produced by		
	LM1+Sucrose	LM1+SUGARCANE JUICE	LM1+LIQUID JAGGERY
Glycerol	+	+	+
Ethylene glycol	+	+	+
Formamide	+	+	+
water	+	+	+
Methyl sulphate	+	+	+
Ethanol	-	-	-
Isopropanol	-	-	-
Acetone	-	-	-
Methanol	-	-	-

+= soluble / - = insoluble

Table 3. Determination of Solubility of dextran produced by isolate LM2

Solubility	Dextran produced from different sucrose source		
	LM2 + STD MEDIUM	LM2 + SUGARCANE JUICE BROTH	LM2 + LIQUID JAGGERY BROTH
Glycerol	+	+	+
Ethylene glycol	+	+	+
Formamide	+	+	+
water	+	+	+
Methyl sulphate	+	+	+
Ethanol	-	-	-
Isopropanol	-	-	-
Acetone	-	-	-
Methanol	-	-	-

+= positive test / - = negative test

cane juice and liquid jaggery could be a cost-effective and sustainable approach to enhance the production of dextran gum by *Leuconostoc mesenteroides*. Further studies are required to optimize the growth conditions and to investigate the properties of dextran gum produced using different sources of sucrose (Fig. 1).

The results showed that the viscosity of dextran produced by both isolates was similar when grown in the standard medium and in medium containing sugarcane juice (Table 1). However, the viscosity of dextran produced by both isolates was significantly lower when grown in medium containing liquid jaggery as a sugar source. Isolate LM1 and LM2 produced dextran with a viscosity range of 0.005-0.01 Pa.s when grown in the standard medium and medium containing sugarcane juice. In contrast, the viscosity of dextran produced by both isolates was less than 0.005 Pa.s when grown in medium containing liquid jaggery. The results of this study suggest that the type of sucrose source used for the growth of *Leuconostoc mesenteroides* significantly affects the viscosity of dextran gum. Liquid jaggery, which is a byproduct of sugarcane juice processing, contains impurities and minerals that may affect the viscosity of dextran produced. The presence of impurities and minerals in the medium may interfere with the polymerization of dextran, leading to the production of dextran with lower viscosity.

Conclusion

The use of natural sugar sources such as sugarcane juice and liquid jaggery could be a cost-effective and sustainable approach to enhance the production of dextran gum by *Leuconostoc mesenteroides*. Further studies are required to optimize the growth conditions and to investigate the properties of dextran gum produced using different sources of sucrose. The more studies should be carried out to explore the potential of isolates of *Leuconostoc mesenteroids* for dextran gum production.

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Conflicts of Interests

The authors declare that there are no conflicts of interests

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