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Research Article

# Effect of Nitrogen Sources and Sucrose Concentration on Dextran Production by *Leuconostoc mesenteroides* NRRL-B-512F

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## Abstract

This study investigated on the production, properties and application of the biopolysaccharide dextran produced via fermentation with *Leuconostoc mesenteroides.* Sucrose broth medium was used for dextran production *Leuconostoc mesenteroides* strain NRRL-B-512F. Six different inoculums were made according to different nitrogen sources in the fermentation medium (Sucrose peptone, glucose peptone, lactose peptone, sucrose ammonium sulphate, glucose ammonium sulphate and lactose ammonium sulphate). Maximum yield of dextran was obtained from glucose ammonium sulphate which gave promising results among six different inoculums. Different sucrose concentrations were used for optimum dextran production which gave promising result. TLC was performed using propanol: water (16:3) as a solvent and Rf value of dextran was found to be 0.2.

**Keywords:** Dextran, *Leuconostoc mesenteroides*, nitrogen sources, Thin Layer Chromatography.

## Introduction

Dextran is an extracellular bacterial polymer of D-glucopyranose with α-1,6 linkage (Jeanes, 1966) in main chain and a variable amount of α-(1,2), α-(1,3), α-(1,4) branched linkages (Sidebotham, 1974; Monsan *et al*., 2001). Dextran is synthesized from sucrose and chains of D-glucose units (Kim and Robyt, 1995). Other workers have also reported the formation of dextran from different strains of bacteria that are primarily *Leuconostoc* strains. Among many dextran producing species, dextran produced by *Leuconostoc mesenteroides* shows excellent results. Hucker and Pederson (1939) were the first who reported the production of dextran from sucrose by strains of *Leuconostoc sp*. Many researchers have described the effects of sucrose concentration, aeration rate, agitation speed, medium pH, incubation temperature (Lazic *et al.*, 1993), nature of the yeast and other nutritional requirements for the production of dextran (Tsuchiya *et al.*, 1952) using different strains of *Leuconostoc mesenteroides*. The name Dextran was first used by Scheilber in 1874, when he found the mysterious thickening of cane and beet sugar juices was caused by a carbohydrate having a positive optical rotation. In 1861, Pasteur had shown that these slimes were caused by microbial action and Van Teighem named the causative bacterium *Leuconostoc mesenteroides* (Pasteur, 1861; Scheilber, 1874; Van Tieghem, 1878). Dextran is biodegradable (Zevenhuizen, 1968), stable for more than 5 years, easily filterable, neutral and is water soluble but insoluble in alcohol.

Dextran is produced by various species of *Leuconostoc*, *Streptococcus* and *Acetobacter*. *Leuconostoc species* are epiphytic bacteria that are wide spread in the natural environment and play an important role in several industrial and food fermentations. *Leuconostoc mesenteroides* is a facultative anaerobe (Veljkivic, 1992), which requires complex growth factors and amino acids (Reiter and Oram, 1982) for their growth and metabolism. *Leuconostoc mesenteroides* is responsible for initiating vegetable fermentation (Pederson and Albury, 1969). Maximum yield of dextran is at 25-30°C and pH required is 7-7.4. Nitrogen source is provided by yeast extract and it is partially hydrolyzed with HCl. There are various applications of dextran in several fields. In Photographic industry, highly purified dextran is used where dextran polymer improve the quality of silver emulsions of photograph. Dextran is used in eye drops such as a lubricant, increases blood sugar level, used in cryo-preservation. It is also used as heparin substitute for anticoagulant therapy, antithrombotic and as a volume expander in anemia. Dextran is also used in chemical industry as an adjuvant, emulsifies carrier and stabilizer. It is also used in cross-linked dextran known as Sephadex used for separation and purification (Goulas *et al*., 2004). In addition to this, dextran is also used in food industry as a thickener in Jam and ice creams (Naessens *et al*., 2005) and also used in bakery products to improve softness, texture and loaf volume and prevents crystallization of sugars (Qader *et al.*, 2005; Purama and Goyal, 2005; 2008).

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## Materials and methods

***Media composition and maintenance of dextrin producing bacteria:*** Sucrose broth medium was used for dextran production. Media composition (Sucrose; 10; Yeast extract; 0.5; Peptone; 0.5; $K_2HPO_4$; 1.5; NaCl; 0.001; $MgSO_4.7H_2O$; 0.001; $CaCl_2$; 0.005 g/100 mL) varied according to different nitrogen sources (Ali, 2005). *Leuconostoc mesenteroides*–NRRL B-512F was procured from NCL, Pune, sub-cultured and maintained on MRS media.

***Production of Dextran:*** Loopful culture of *Leuconostoc mesenteroides* was inoculated in 5 mL sterile fermentation media at 26°C for 24 h. About 5 mL of 24 h old culture was then transferred into 45 mL of sterile broth medium and incubated again at 26°C for 24 h. This 50 mL inoculum was used for dextran production. Six different inoculums were made according to different nitrogen sources in fermentation medium namely Sucrose peptone, glucose peptone, lactose peptone, sucrose ammonium sulphate, glucose ammonium sulphate and lactose ammonium sulphate. About 450 mL of sterile fermentation medium was inoculated with 50 mL of inoculum and incubated at 26°C for 18 h. The culture medium becomes very viscous as the pH of the medium drops from 7.5-5.5 during the fermentation period.

***Precipitation of dextran:*** The culture medium after 18 h of incubation was precipitated using chilled ethanol. In the first step, equal amount of ethanol was added to it, stirred well and was further centrifuged. The supernatant was then decanted and pellet was used for further process. In the second step, chilled ethanol was added with constant stirring and precipitation of dextran was observed. It was allowed to stand for 5-10 min and supernatant was again decanted. After standing 10 min, chilled ethanol was added and dextran was precipitated in very fine form. The cells were dried in an oven at 80°C and the dry weight was calculated.

***Thin Layer Chromatography:*** TLC of dextran was performed using propanol and water (16:3) as solvents. Rf value was calculated and compared with the standard Rf value of dextran.

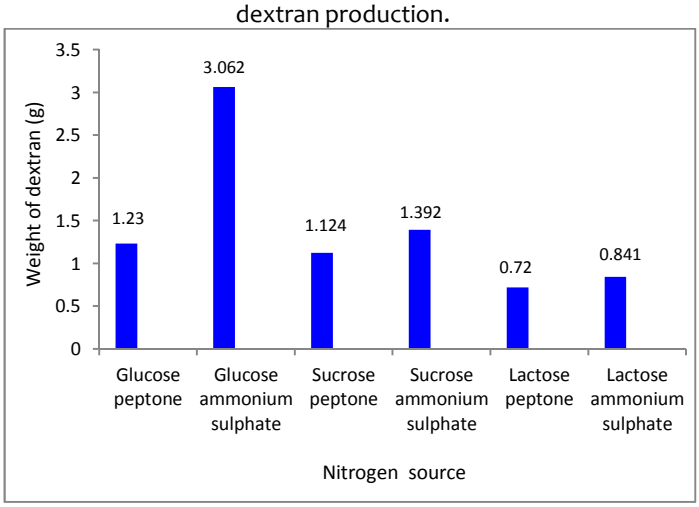
## Results and discussion

Dextran is a sucrose dependent polysaccharide which contains a backbone of α-D glucose. Dextran is used for different purposes in cryopreservation and storage of organs for transplantation and as carriers in vaccines. Because of its favorable product characteristics, technical grade dextran serves as important starting and intermediate reagents in a broad range of synthesis in biotechnological and technical industries.

Table 1. Effect of different nitrogen sources on dextran production.

| Nitrogen source | Weight of dextran (g) |
|---|---|
| Glucose peptone | 1.230 |
| Glucose ammonium sulphate | 3.062 |
| Sucrose peptone | 1.124 |
| Sucrose ammonium sulphate | 1.392 |
| Lactose peptone | 0.720 |
| Lactose ammonium sulphate | 0.841 |

Fig. 1. Effect of different nitrogen sources on dextran production.




Dextran has been extensively studied because of their actual and potential application in food industry, agricultural industry, pharmaceutical and chemical industries. Dextran 70 is generally marketed as a 6% solution in a normal saline and used as a plasma volume expander. It is recommended for the treatment of shock for hemorrhage, burns, surgery or trauma. It also reduces the risk of thrombosis. In this study, dextran production was carried out using *Leuconostoc mesenteroides*. The bacterial species was then provided with a fermentation medium for high yield of dextran and was further extracted. The effect of substrate concentration on dextran production plays an important role. Different nitrogen sources were used for the optimization of dextran production. Among them, glucose ammonium sulphate was found to be the excellent nitrogen source as it has given the maximum yield of dextran (Table 1 and Fig. 1). For maximum dextran production, it is necessary that optimal amount of sucrose in the fermentation media should be provided. As the concentration of sucrose in the fermentation medium increases, the dextran production also increases rapidly (Table 2). Results have shown that 30% sucrose was optimum for dextran production (Fig. 2). It was also observed that if sucrose concentration exceeds 30%, then there was a decrease in the percentage conversion of sucrose to dextran.

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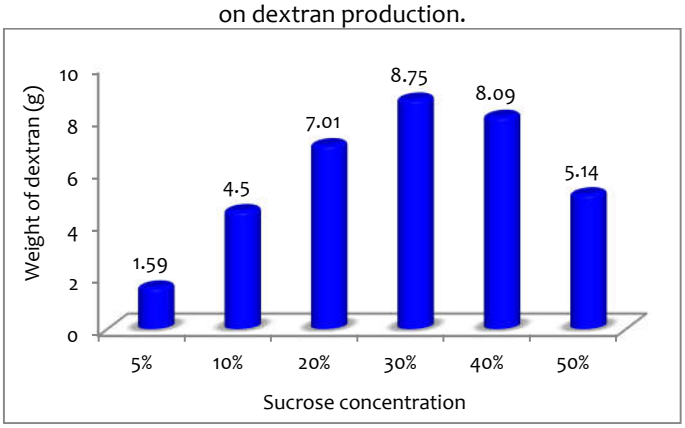
Table 2. Effect of different concentrations of sucrose on dextran production.

| Sucrose (%) | Weight of dextran (g) |
|---|---|
| 5% | 1.59 |
| 10% | 4.50 |
| 20% | 7.01 |
| 30% | 8.75 |
| 40% | 8.09 |
| 50% | 5.14 |

Fig. 2. Effect of different concentrations of sucrose on dextran production.




Higher sucrose concentration has an inhibitory effect which decreases the dextran production (Martinez-Espindola and Lopez-Manguia, 1985). Similar study was carried out by Qader (2005) where production of dextran was carried out using newly isolated strains of *Leuconostoc mesenteroides* PCSIR-4 and PCSIR-9. Estimation of dextran was done by Thin layer Chromatography and Rf value of dextran was found to be 0.2.

## Conclusion

From this study it may be concluded that there is a need for optimizing the process parameters for dextran production. It was found that dextran production was maximum at 30% sucrose concentration and glucose ammonium suphate acted as the best nitrogen source. Rf value of extracted dextran was compared with the standard value of dextran and it was found to be 0.2.

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