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Production of Dextran by Newly Isolated Strains of *Leuconostoc mesenteroides* PCSIR-4 and PCSIR-9

[Yeni Ayrıştırılmış *Leuconostoc mesenteroid PCSIR-4 ve PCSIR-9* Suşlarında Dekstran Üretimi]

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ABSTRACT

Different isolated strains of Leuconostoc mesenteroides produce dextran compared with that of *L. mesenteroides* NRRL B-512 F. Among nine newly isolated strains, strains PCSIR-4 and PCSIR-9 produced dextran of different quality. Using these two newly isolated strains, the development of dextran coincided closely with the disappearance of sucrose. The yield of dextran increased during growth and maximum yield was obtained at the end of exponential phase. Dextran yield was higher in media containing nitrogen source supplemented with different salts. Medium 2 was found to be best for the dextran production as compared with other media. Initial sucrose concentration in the media plays an important role in dextran production. The higher the initial concentration of sucrose in the medium, the higher is the yield of dextran produced per unit volume; however, the percentage conversion of sucrose into dextran decreases after reaching maxima. A 10% sucrose concentration in the medium was the optimal level of dextran production. Molecular mass distribution of dextran from PCSIR-4 is near to 2 million Dalton whereas the dextran produced by PCSIR-9 is quiet higher than the dextran from PCSIR-4.

Key Words: Dextransucrase, dextran, polysaccharide, *Leuconostoc mesenteroides*

ÖZET

Leuconostoc mesenteroid'in L. mesenteroides NRLL B-512'den farklı ayrıştırılmış suşları dekstran oluşturur. Yeni ayrıştırılan dokuz suştan PCSIR-4 ve PCSIR-9 farklı özellikte bir dekstran üretir. Yeni ayrıştırılan bu suşları kullanarak, dekstran gelişimi sukroz yitimi ile eş zamanlı olmuştur. Dekstran verimi büyüme aşamasında artmış ve logaritmik büyüme aşamasının sonunda en fazla verim elde edilmiştir. Dekstran verimi farklı tuzlar ile zenginleştirilmiş azot kaynağı içeren besiyeri ile daha yüksek bulunmuştur. Diğer besiyerleri ile larşılaştırıldığında besiyeri-2'nin dekstran üretimi için en uygun olduğu saptanmıştır. Besiyerindeki başlangıç sukroz derişimi dekstran üretiminde önemli role sahiptir. Ortamdaki başlangıç sukroz derişimi ne kadar yüksekse birim hacim başına üretilen dekstran verimi de o kadar yüksektir; ancak sukrozun dekstrana dönüşüm yüzdesi en üst seviyeye ulaştıktan sonra düşmektedir. Besiyerinde % 10 sukroz derişimi dekstran üretimi için en uygun derişim olarak bulunmuştur. PCSIR-4'ün ürettiği dekstran yaklaşık 2 milyon Dalton moleküler ağırlık dağılımına sahipken, PCSIR-9 kökenli dekstran PCSIR-4 kökenli dekstrandan daha ağırdır.

Anahtar Kelimeler: Dekstransukraz, dekstran, polisakkarid, *Leuconostoc mesenteroid*

Introduction

Dextran is a group of high molecular mass polysaccharides that are synthesized from sucrose and composed of chains of D-glucose units [7]. Dextran is produced by species of Leuconostoc, Streptococcus and Acetobacter. Hucker and Pederson [5] was the first who reported the production of dextran from sucrose by strains of Leuconostoc species. Jeanes et al. [6] reported the formation of dextran from different strains of bacteria that were primarily Leuconostoc strains. Species of bacteria from other genera have been also found to produce dextran. In 1941 [13] dextran production from sucrose by Streptococcus species was reported. Hehre and Hamilton [3] compared the dextran produced by Acetobacter species and found them similar to that of Leuconostoc species. The most commercially used strain of *L. mesenteroides* is NRRL B- 512F, which produces, water soluble dextran containing 95% linear α-(1→6) linkage and 5% α-(1→3) linkage [15]. Dextran is of particular interest because of its uses as blood-plasma volume expender. It finds various other industrial applications in food, pharmaceutical and chemical industries as adjuvant, emulsifier, carrier and stabilizer. Cross-linked dextran known as sephadex are widely used for separation and purification of various products like protein in research and industry. In food industry it is being used as thickener for jam and ice cream. It prevents crystallization of sugar, improves moisture retention, maintain flavor and appearance of various foodstuffs. Due to its numerous industrial application it is being produced commercially using the strain of *L. mesenteroides NRRL B-512F.* Present study is an attempt to establish conditions for optimum production of dextran by two newly isolated strains of *L. mesenteroides.* The results have been compared with the reference strain NRRL B-512F

Materials and Methods

The strains of *Leuconostoc mesenteroides* were isolated from the fermented vegetables such as cabbage, cauliflower, pumpkins, carrot and tomato in sucrose broth medium [14], and identified by usual methods of Holt [4]. All these strains were maintained on sucrose broth medium at 4°C. The reference strain of *L. mesenteroides NRRL B-512F* was received from Prof. A. Lopez Munguia (Mexico University, Mexico).

Preparation of inoculum

10 ml of sterile sucrose broth was innoculated by a loopful of growing culture of *L. mesenteroides* at 26°C for 24 h, and 10 ml of 24 h old culture was then transferred into 90 ml of sterile broth medium and incubated again for 24 h at 26°C. This 100 ml innoculum was used for dextran production

Production of dextran

Sterile sucrose broth medium (900ml) was innoculated with 100 ml of innoculum and incubated at 26°C for 18 h. This culture medium became very viscous and pH of the medium dropped from 7.5 to 5.5 during the fermentation.

Precipitation of dextran

The culture medium after 18 h of incubation was precipitated by using chilled ethanol. In the first step, equal amount of ethanol was added and stirred well and centrifuged. The supernatant was decanted. In the second step, chilled ethanol was added with constant stirring and precipitates of dextran appeared. It was allowed to stand for 5–10 minutes and supernatant was again decanted. After standing 10 minutes chilled ethanol was added again and dextran was precipitated in very fine form. The precipitated dextran was dried under vacuum over calcium chloride at 30°C. The yield was calculated on dry weight basis.

Purification of dextran

In 10 g of dextran 200 ml cold water was added, 100 ml water was added step wise to make a paste of dextran in water. Dextran was precipitated with chilled ethanol. This cycle of redissolving, precipitation and washing was repeated three times. The dextran dried under vacuum over calcium chloride at 30°C.

Enzyme assay

The enzyme activity was determined in the cell free supernatant by the method of Kobayashi and Matsuda [8]. Units of Enzyme activity are represented in DSU/ml/hr [9].

"One unit of enzyme activity was defined as the enzyme quantity that converts one mg of sucrose into fructose and dextran in one hour under the condition"

Estimation of total protein

Total protein of the cell-free filtrate was determined by the method of Lowry [10]. Bovine serum Albumin was used as a standard curve ranging from 25 µg/ml to 250 µg/ml.

Determination of average molecular weight of dextran

The average molecular weight of dextran produced by fermentation and immobilization technology from *L. mesenteroides* was determined by gel permeation chromatography on LKB gel filtration system using blue dextran 2000 (average Mol.wt. 200,000) as standard. The sample was applied through automatic sample applicator on XK16/70 glass column packed with sephacryl-S-200HR. It was eluted with 0.05 M phosphate buffer (pH 7.0) at a constant flow rate of 20 ml/hr. The fractions (40 drops/ Fr.) were collected through automatic fraction collector Ultro Rac II (Model LKB 2070). All dextran fractions were eluted in void volume.

Table 1. Media composition for dextran production from *L. mesenteroides* PCSIR- 4 and PCSIR-9

Ingredients (g/100ml)	Medium			
	1	2	3	4
Sucrose	10.000	10.000	10.000	10.000
Yeast extract	0.500	0.500	0.500	2.000
Peptone	0.500	0.500	0.500	—
K_2HPO_4	0.500	1.500	—	—
NaCl	—	0.001	—	0.001
$MgSO_4.7H_2O$	—	0.001	—	0.001
$MnCl_2.H_2O$	—	0.001	—	—
$FeSO_4.7H_2O$	—	—	—	0.001
$CaCl_2$	—	0.005	—	0.005

Initial pH values of the media were adjusted to 7.5 before sterilization

Determination of viscosity

Viscosity of fermentation media and dextran solution was determined with a standardized Ostwald viscometer tube at 26°C.

Results and Discussion

The source of nitrogen and other nutrients in fermentation medium play an important role in rate of growth and dextran production. Table 1 shows that different media contain different types of carbon and nitrogen source, which affect the conversion of sucrose to dextran by growing microorganism. McClesky et al. [12] reported that composition of medium had a pronounced effect on dextran production. Table 2 shows the production of dextran on different media containing different salt composition. Dextran production and enzyme activity was found to be maximum in medium 2, which was enriched with various salts. Table 3 shows production of dextran and enzyme activity from different locally isolated strains with reference to NRRL B-512F. It was observed that locally isolated strain PCSIR-4 and PCSIR-9 shows extraordinary behavior. Among all isolated strains, PCSIR-4 exhibited the maximum dextran while PCSIR-9 shows a thick and highly viscous dextran that indicates a high molecular mass. Table 4 indicates the time course of dextran production by the *L. mesenteroides* PCSIR-4 and PCSIR-9 with comparison with NRRL

Table 2 Effect of media composition on dextran production from *L. mesenteroides* PCSIR-4 and PCSIR-9

Medium	PCSIR-4		PCSIR-9	
	Dextran (g)	Viscosity (cp.)	Dextran (g)	Viscosity (cp.)
1	3.68	23.15	3.09	19.21
2	4.78	26.87	3.79	41.56
3	3.40	18.72	3.12	18.56
4	3.96	25.08	3.16	27.35

Table 3. Dextran production and enzyme activity from various newly isolated strains of *L. mesenteroides* with reference to NRRL B-512F

Serial No	Strains of *L.mesenteroides*	Enzyme activity (DSU/ml)	Dextran (g)	Percent conversion of sucrose
1	PCSIR-1	27.91	3.50	35.00
2	PCSIR-2	16.65	3.20	32.00
3	PCSIR-3	14.13	3.45	34.50
4	PCSIR-4	108.26	4.78	47.80
5	PCSIR-5	41.05	3.25	32.50
6	PCSIR-6	35.03	3.40	34.00
7	PCSIR-7	24.08	3.60	36.00
8	PCSIR-8	32.29	3.92	39.20
9	PCSIR-9	27.63	3.79	37.90
10	NRRL B-512F	35.57	4.08	40.80

Table 4. Time course of dextran production at 10% (w/v) sucrose from *L. mesenteroides* PCSIR-4 and PCSIR-9 with reference to NRRL B-512F.

Incubation time (hrs)	Dextran (g)		
	PCSIR-4	PCSIR-9	NRRL B-512F
2	1.27	1.37	1.26
4	1.30	1.58	1.54
6	2.16	2.94	2.61
8	3.72	3.23	2.71
12	4.12	3.68	3.00
18	4.78	3.79	4.08
24	4.49	3.02	4.00
48	4.48	3.02	3.69
72	4.31	3.00	3.35

Table 5. Time course of cellular growth, pH, enzyme activity and protein by *L. mesenteroides* PCSIR-4 grown in media containing 2% (w/v) sucrose

Incubation time (h)	Final pH	Wet cell mass (g/dl)	Enzyme activity (DSU/ml)	Total protein (mg/dl)
0	7.00	0.39	—	210
2	6.90	0.52	—	400
4	6.65	0.62	7.66	260
6	5.92	0.74	16.42	232
8	5.80	0.75	21.90	203
12	5.63	0.74	50.30	203
18	5.56	0.96	108.26	186
24	5.54	0.98	38.31	182
48	5.54	0.91	35.57	184
72	5.54	0.80	27.36	203

Table 6. Time course of cellular growth, pH, enzyme activity and protein by *L. mesenteroides* PCSIR-9 grown in media containing 2% (w/v) sucrose

Incubation time (h)	Final pH	Wet cell mass (g/dl)	Enzyme activity (DSU/ml)	Total protein (mg/dl)
0	7.10	0.17	—	175
2	6.80	0.39	—	405
4	6.74	0.60	—	300
6	6.15	0.79	3.60	253
8	5.78	0.71	7.20	225
12	5.76	0.90	10.13	220
18	5.70	1.44	27.63	209
24	5.70	1.10	9.45	196
48	5.65	0.99	8.35	200
72	5.65	0.96	5.32	196

B-512. Maximum dextran production was obtained after 18h and remained nearly constant but slightly decreasing up to 72h. Zedan et al. [16] reported that fermentation of sucrose for the formation of dextran and fructose was performed by dextransucrase of the culture, which is continuously produced from multiplication of bacteria. Dextran production increases from 4h of incubation to 24h. On further incubation dextran production does not increases. Table 5 and Table 6 also supported the idea that dextran production is associated with exponential phase and early stationary phase of bacteria and, when decline phase of *L. mesenteroides,* is reached, no dextran production takes place and production becomes stable. Baily and Oxford [1] reported that maximum dextran yield of *Streptococcus bovis* is obtained during the continues production of enzyme, which ultimately is produced during growth of bacteria. PCSIR-4 and PCSIR-9 shows maximum dextran production after 18 hours. The effect of substrate concentration on the dextran production plays an important role. 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 media increases, the dextran production increases. Results shows that 10% sucrose was optimal level for dextran production as reported earlier [2]. It was also observed that if sucrose concentration is higher than 10% , there was a decrease in percentage conversion of sucrose in dextran. Higher sucrose concentration has an inhibitory effect and this effect is called substrate inhibitory effect, which decreases the dextran production in higher concentration of substrate [11]. This decrease related

Table 7. Effect of sucrose concentration on dextran production by *L. mesenteroides* PCSIR-and PCSIR-9 in fermented media

Sucrose g/100ml	PCSIR-4			PCSIR-9			NRRL B-512F		
	Dextran (g)	Percentage conversion	Viscosity (cp.)	Dextran (g)	Percentage conversion	Viscosity (cp.)	Dextran (g)	Percentage conversion	Viscosity (cp.)
5	1.79	35.80	11.25	1.57	31.40	10.39	1.70	34.00	4.73
10	4.78	47.80	26.87	3.79	37.90	41.56	4.08	40.80	22.03
20	7.01	35.05	28.66	5.87	29.35	31.89	5.46	27.30	31.89
30	9.04	30.13	20.64	5.74	19.13	20.06	5.45	18.17	27.59
40	8.09	20.23	24.00	4.57	11.43	22.93	4.46	11.15	24.00
50	5.32	10.63	10.75	3.65	7.30	21.14	3.12	6.24	18.59

Table 8. Effect of sucrose concentration on the quality of dextran produced in fermented media

S. No	Sucrose (g)	Product Feature		
		PCSIR-4	PCSIR-9	NRRL B-512F
1	5	Very fine powder	Fine powder	Fine Powder
2	10	Very fine powder	Granular	Granular
3	20	Fine powder	Granular	Granular
4	30	Granular	Granular	Granular
5	40	Granular	Granular	Granular
6	50	Granular	Granular	Granular

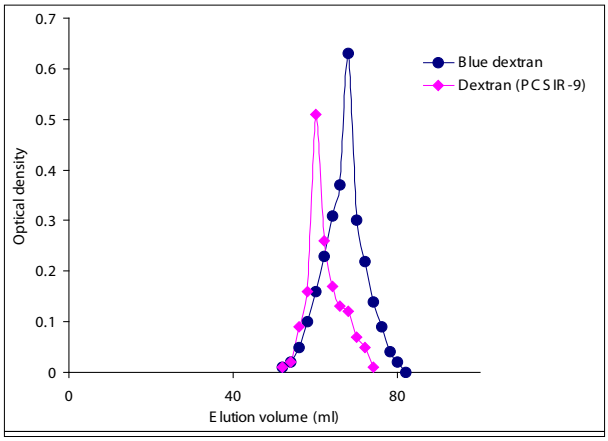

Figure 2. Molecular mass distribution of Dextran from Leuconostoc mesenteroides (PCSIR-9) with reference to Blue dextran

to the size of the polymer chain and their interaction with the solvent. The polymer synthesized at a sucrose concentration higher than optimum, produce dextran of high molecular mass. For which polymer-polymer and polymer-solvent interactions are more significant. Due to these interaction the physical characteristic of dextran changed significantly. Table 8 shows physical characteristic of dextran produced by PCSIR-4 and PCSIR-9 with reference to NRRL B-512F. It was observed that very fine quality dextran was obtained at 10 % sucrose concentration from PCSIR-4 and as the sucrose concentration increases, granular dextran was obtained. Dextran from PCSIR-9 and NRRL B-512F was fine quality but it was not so fine as it was from PCSIR-4. Dextran from PCSIR-9 and NRRL B-512F was granular at 10 % sucrose concentration in media due to the production of high molecular mass (Fig. 3). Isolated strains of *Leuconostoc mesenteroides* PCSIR-4 and PCSIR-9 shows different molecular mass distribution. Fig 1 shows that PCSIR-4 produces dextran of same molecular mass with the reference to blue dextran. Elution pattern clearly indicates that peaks of the dextran from *L. mesenteroides* PCSIR-4 is overlapping the peak of Blue dextran, which indicates that the molecular mass of PCSIR-4 dextran is about 2 million Dalton. Fig. 2 shows the molecular mass distribution of dextran from *L. mesenteroides* PCSIR-9. Elution pattern clearly indicates that dextran is of high molecular mass as compared to blue dextran. This result is also supported by Table 2 and Table 7 in which viscosity of dextran is very much higher as compared to other dextran. Fig. 3 shows the comparison of molecular mass of dextran from PCSIR-4 and PCSIR-9 and we found that these two strains produce different molecular mass of dextran. Molecular mass of dextran from PCSIR-4 is low as compared to dextran from PCSIR-9. *L. mesenteroides PCSIR-9* produces high molecular mass dextran with very high viscosity (Fig. 3).

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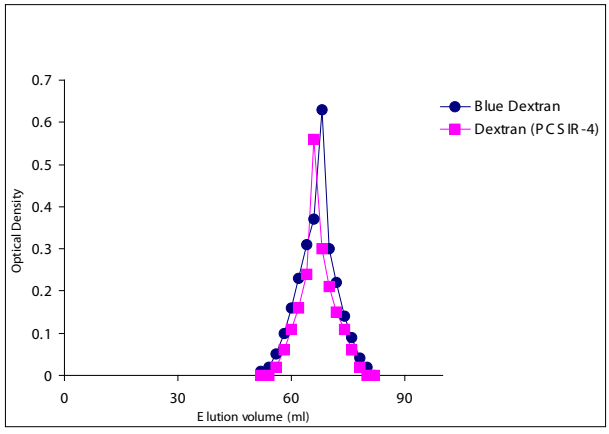

Figure 1. Molecular mass distribution of Dextran from Leuconostoc mesenteroides (PCSIR-4) with reference to Blue dextran

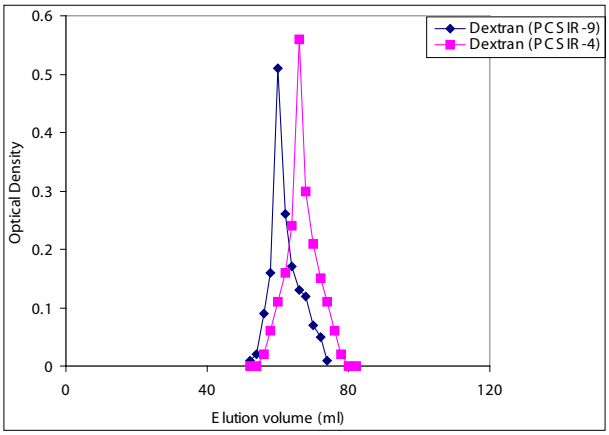

Figure 3. Molecular mass distribution of Dextran from *L. mesenteroides* PCSIR-9 and PCSIR-4

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