Characterization of Dextran Produced by *L. mesenteroides* ATCC 13146

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Abstract

High molecular weight dextran (39% alcohol, v/v), less soluble dextran, eluted from this column between T 500 and T 2000, a commercial linear dextran. Soluble dextran (45% alcohol, v/v) eluted at between T 70 and T 150 dextran. The molecular weight average of total dextran (50% alcohol, v/v) was between 150,000 to 500,000. A few oligosaccharides were detected from hydrolyzates of less soluble dextran. The hydrolyzates of soluble dextran were a family of DP 1 to 6 isomaltooligosaccharides. Compounds greater than DP 4 were branched isomaltooligosaacharides.

Introduction

Functional foods are defined as any modified foods and food ingredients that provide health benefits beyond the traditional nutrients they contain (Haseler, 1996). Non- or partially digestible dextran as a dietary fiber are one of the most popular functional food components. *Leuconostoc mesenteroides* ATCC 13146 produces two different structural types of dextrans. The S dextran consists of 67% of α -D-(1-6) of main chain , 24% of α -D-(1-4) branches, and 9% of α -D-(1-3) branches. The L dextran fraction was 79% of α -D-(1-6) of main chain, 20% of α -D-(1-4) branches, and 2% or less of α -D-(1-3) branches. The present study was conducted to determine the average molecular weight and hydrolysis by hydrolytic enzyme of dextran produced by *Leuconostoc mesenteroides* ATCC.

Materials and methods

Bacterial strain

Leuconostoc mesenteroides ATCC 13146 was purchased from American Type Culture Collection (Rockville, MD, USA). Dextran production medium consists of 3.0 g K_2HPO_4, 0.01 g $FeSO_4.H_2O$, 0.01 g $MnSO_4$. $7H_2O$, 0.01 g NaCl, 0.05 g $CaCl_2$, 0.5 g yeast extract, 15 g agar and 150 g sucrose per liter deionized water. Medium pH was adjusted to 6.0 prior to sterilization. Dextran was produced in 2L-bioreactor at 25 °C and 200 rpm for 24 h.

Molecular weight determination

Dextran size was determined using gelpermeation chromatography with Ultrahydrogel Linear column (Millipore, MA). Deionized water was the eluent and the temperature was ambient The rate of elution was 1.0 mL / min. The detector was a 410 Differential Refractometer (Millipore Corporation, MA). Dextran sizes were calculated from a standard curve made using the following standards (0.1%, w/v); T 5, T 10, T 40, T 70, T 500, T 2000 (Pharmacia, Piscataway, NJ). Sample concentration was 0.5% (w/v) and it was passed through a 0.2 mm filter prior to use.

Dextran and oligosaccharide hydrolysis

Dextrans were hydrolyzed by two dextranases produced by *L. starkeyi* and *Penicillium* (Sigma Chemical Company, MO). Dexytans, 0.5% (w/v), were dissolved in 2 mL of acetate buffer containing 100 mM sodium acetate, 20 mM CaCl2, and 0.02% (w/v) sodium azide. The solution was adjusted to pH 5.2 and then Penicillium dextranase (0.3 IU / mL) or L. starkeyi (0.5 IU / mL) dextranase was added and incubated with at 35C for 24 to 48 hrs. Hydrolyzates were analyzed by either TLC or HPLC.

Results

The range of molecular weights of the dextrans were determined by gel permeation chromatography, Ultrahydrogel Linear column (Millipore).

High molecular weight dextran (39% alcohol, v/v), less soluble dextran, eluted from this column between T 500 and T 2000, a commercial linear dextran (Table 1). Soluble dextran (45% alcohol, v/v) eluted at between T 70 and T 150 dextran. The molecular weight average of total dextran (50% alcohol, v/v) was between 150,000 to 500,000. Dextran produced by ATCC 13146, T 500, and dextran produced by ATCC 18010 were hydrolyzed by dextranase produced by a Penicillium sp. or *L. starkeyi* ATCC 74054. The soluble (45%) dextran, less soluble (39%) dextran, total dextran (50%) produced by ATCC 13146 were all more resistant to dextranase than the dextrans produced by ATCC 18030 (Table 2).

The degree of hydrolysis of dextran by Lipomyces dextranase was greater than that of Penicillium dextranase. Dextran hydrolyzates were analyzed by TLC (Fig. 12). Lipomyces dextranase released mostly glucose (36.7% hydrolysis) from less soluble dextran (Lane A1, 2, and 3). This dextran was resistant to Penicillin dextranase (3.9% hydrolysis).

Table 1. Distribution of dextran size produced by *Leuconostoc mesenteroides* ATCC 13146.

Dextrans	Molecular Weight	Elution volume (mL/min)
Linear dextrans [a]		
T 2000	2,000,000	7.651
T 500	500,000	7.767
T 150	150,000	8.067
T 70	70,000	8.283
T 40	40,000	8.517
T 10	10,000	9.142
T 5		
Dextrans (ATCC 13146)		
Dextrans (50%)[b]	150,000 to 500,000	7.852
Less soluble (39%)	> 500,000	7.365
Soluble (45%)	70,000 to 150,000	8.125

[a] Commercial dextrans were obtained from Pharmacia Co. and Sigma Co.

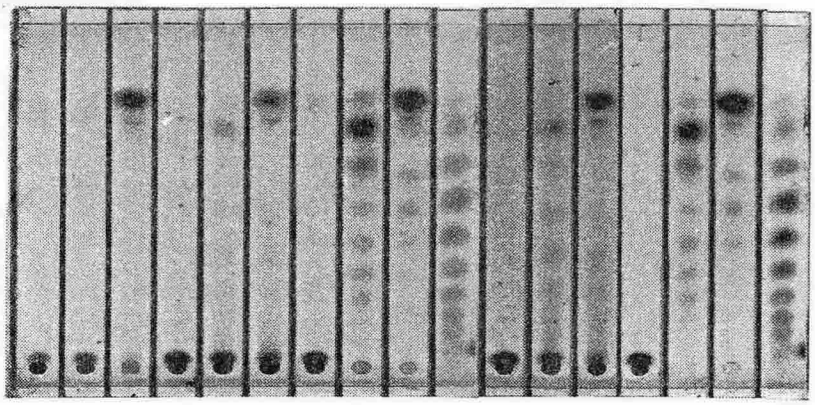
[b] Dextrans were fractionated by ethanol.

Table 2. Hydrolysis of dextran produced by *Leuconostoc mesenteroides* ATCC 13146.

Dextrans	% Hydrolysis	
	Penicillium Dextrnase	Lipomyces Dextranase
Dextran (ATCC 13146)	11.96	36.39
Less soluble (39%)	3.94	36.73
Soluble (45%)	6.12	21.52
T 500	38.37	55.49
Dextran (ATCC 18030)	36.50	55.58

Dextranase (0.5 IU) produced by Penicillium (Sigma) and Lipomyces was reacted with 0.5% (w/v) dextran solution at condition of pH 5.2, 100 mM sodium acetate, 35°C, and 48 hr reaction.

A few oligosaccharides were detected from hydrolyzates of less soluble dextran. The hydrolyzates (B1, 2 and 3) of soluble dextran were a family of DP 1 to 6 isomaltooligosaccharides. By comparing the Rfstvalues with those of linear standard isomaltooligosaccharides {a-D-(1-6)-linked D-glucopyranosyl residues} (Lane S), the hydrolyzates of soluble dextran might contain a-D-Glc (1-4)- or Glc (1-3) - branched linkages on a-D-Glc (1-6)- main chain. Penicillium dextranase gave a 3.5 fold higher hydrolysis to the soluble dextran than by Lipomyces starkyei ATCC 74054 dextranase. The enzymatic hydrolyzates of total dextran of ATCC 13146 (D1, 2, and 3) and ATCC 18030 (E1, 2, and 3) showed different patterns. Hydrolyzates of ATCC 13146 (Lane D2) branched dextrans by Penicillium dextranase contained of glucose, isomaltose, isomaltotriose, and isomaltooligosaccharides greater than DP4 that had branch points. Compounds greater than DP 4 were branched isomaltooligosaacharides. Hydrolysates by the Lipomyces dextranase produced the same composition of oligosaccharides as those by Penicillin dextranase (Lane D3). The hydolysates of ATCC 18054 dextran (Lane E2 and 3) and a linear dextran of T 500 (Lane C2 and 3) produced an isomaltooligosaccharide distribution.



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