Pakistan J. Sci. Ind. Res., Vol. 17, No. 6, December 1974

PREPARATION AND CHARACTERIZATION OF DEXTRAN FROM SUCROSE BY THE ACTION OF A LOCALLY ISOLATED STRAIN OF LEUCONOSTOC MESENTEROIDES

Mrs. Jameela Jafri, (Miss) Rabia Zuberi and S. Ali Hasnain Zaidi

PCSIR Laboratories, Karachi 39

(Received July 3, 1974; revised October 22, 1974)

Abstract. The preparation and characterization of a water-soluble dextran from sucrose by the action of a locally isolated strain of *Leuconostoc mesenteroides* has been studied. The production of dextran in the culture medium reached a maximum of 58% of the available glucose from sucrose in 18 hr. About 68% available fructose was present in the filtrate of the culture medium after removal of the dextran. Extracts of 1 g rice polishing or 0.5 g wheat bran respectively were found to be sufficient for both the growth of the bacteria and production of dextran from 10 g sucrose in 100 ml culture medium.

The bacterial polysaccharide, dextran, has been known for a long time.[1] It is a branched polymer in which an organizing unit of five D-glucose residue is repeated throughout the molecule. It has a high molecular weight depending upon the strain and method used for its preparation. Industrial dextran may have a mol.wt. of approximately 40,000,000 but it can be degraded to low mol. wt. fragments by enzymes or acids. It finds many uses in the pharmaceutical and food industries. Partially hydrolysed or degraded dextran is used as a plasma volume-expander. A low molecular dextran–iron complex is used in the treatment of iron-deficiency anaemia. It can be used for the preparation of non-crystallizing sugar syrups of exceptionally high viscosity. It has also been used as a partial substitute for barley malt.

It is generally recognized that dextran is of variable composition. Numerous factors such as composition of the culture medium, aeration and incubation time appear to influence the properties and the yield of dextran and of the byproducts produced from sucrose by culture of *L. mesenteroides*. A factor of outstanding importance is, however, the strain[2–4] of the organism, which appears to determine the properties of the dextran. The present paper, describes the preparation and characterization of dextran from sucrose by the action of a locally isolated strain[5] of *L. mesenteroides*. Observations are also reported here on the effect of the composition of the medium, temperature and incubation time on the yield of this dextran.

Experimental

Organism. The strain of *L. mesenteroides* was isolated from the fermented vegetables[5] such as cauliflower, cabbages, pumpkin and tomato. It was identified by the usual methods.[6]

Media. The composition of the medium used for the growth of the *L. mesenteroides* strain and for the preparation of dextran was (g/100 ml) sucrose 10, tryptone 1.0, yeast extract 0.5, sodium chloride 0.1 and dipotassium hydrogen phosphate 0.1. The pH was adjusted to 8 with NaOH and sterilization was effected by autoclaving at 15 lb pressure/in^2 for 15 min.

Preparation of Inoculum. A culture of the organism was prepared by inoculating 10 ml sterile medium in a test-tube with one loopful of rapidly growing stock culture. It was incubated at 25°C for 18 hr and again transferred to 90 ml sterile medium in a 250 ml flask and was incubated at 25°C for a further 18 hr. This culture, totalling 100 ml, was mixed with 900 ml sterile medium and allowed to stand at 25° for 18 hr. It was used for the preparation of dextran. The preferred incubation time of 18 hr was adopted on the basis of the experimental observation that when 18-hr inocula were used, the yield of dextran produced in the culture medium reached a maximum in 18 hr.

Preparation of Dextran. The medium (900 ml) were sterilized in a 2-litre flask and inoculated with 100 ml 18-hr inocula. It was incubated at 25–27°C. The fermentation process was followed by determining the pH and viscosity of the culture medium after 2, 4, 6, 12, 18 and 24 hr incubation time. The culture medium became viscous and pH dropped to 4.5 during the fermentation. At the completion of the process the viscous material appeared to be homogenously disperesed in the culture medium and did not settle out. The viscosity, pH of the culture medium and the yield of the dextran are given in Table 1. 500 ml 20% aqueous alcohol was added to the culture medium, stirred vigorously and centrifuged twice. The supernatant was decanted off, cooled to 15°C and 95% alcohol was added slowly with mechanical stirring till its concentration became 50%. It was allowed to stand for 5 minutes and the supernatant was decented off from the precipitated dextran. Another 500 ml alcohol was added to the precipitate and stirred. After standing for about 10 min the supernatant was decanted off and another 200 ml alcohol was added to the flocculent precipitate and stirred again. After decanting the supernatant, this mass was filtered under vacuum. It was washed with 100 ml alcohol in small portions during filtration. Dextran was further dried under vacuum ($CaCl_2$) at room temperature. The wt of the product (dry basis) was 300 g., which was equivalent to 30% of the initial wt of sucrose or 60% glucose available from the sucrose.

Purification. Cold water 15°, 200 ml was added to 100 g dextran to make a paste. More water was

added in portions to the stirred paste so as to get a homogeneous (2.5% w/v) solution. The solution was centrifuged twice and the dextran was precipitated from the supernatant with alcohol as before. This cycle of redissolving, precipitating and washing was repeated twice more. The wt of the purified product was (dry wt basis) 96.5 g. This is equivalent to 29% of the initial sucrose or 58% glucose available from sucrose.

Composition of the Filtrate. The filtrate and the washings obtained from the culture medium containing 100 g sucrose substrate were evaporated at 40° *in vacuo*. A solid residue (48 g) was obtained. It contained 31 g fructose or 62% fructose available from the sucrose and 2.6% nitrogen. A small amount of low-molecular dextran and traces of glucose were also present in the residue.

Preparation of Rice-Polishing and Wheat-Bran Extract. The imported tryptone and yeast extract were used in the media to meet the protein and vitamin requirements of the bacteria. Rice-polishing and wheat-bran, abundantly available in this country, contain adequate quantities of both protein and vitamins. Their extracts were, therefore, prepared and used in the media instead of the imported materials. 175 g fresh rice-polishing were placed in a 1-litre flask together with 300 ml water. The pH was adjusted to 3 with HCl and the flask was kept for 6 hr on a water-bath at 60°. The contents were stirred occassionally. After keeping over-night at room temperature (31°) the supernatant was decanted off and filtered over fritted glass. It was used as such. Wheat-bran was also extracred in the same way but the solution was clarified by mixing with 10 g washed fullers earth before filtration through sintered glass funnel. The clear solution was used as such in the media.

Analytical Methods

Chemicals. Tryptone and yeast extract were of bacto grade (Difco). Rest of the chemicals were of A.R. grade (B.D.H.).

Moisture Content. The moisture content was determined on separate samples. Each sample was dried ($CaCl_2$) at room temperature under reduced pressure.

Dextran Contents. The dried sample was estimated for total carbohydrates colorimetrically using anthrone[7] as the test reagent. 0.002% aqueous dextran, grade C, mol wt 60,000 to 90,000, was used as standard.

Reducing Sugars. Reducing sugars were determined colorimetrically by Folin Wu[8] method. 0.2% glucose was used as standard.

Fructose Content. Qualitative test for fructose[3] was made by placing dry dextran in orthophosphoric acid (85–88%) at room temperature. Under these conditions, fructose and sucrose developed dark brown to black colour within 24 hours, but neither glucose nor dextran produced any colour.

Viscosity. Viscosity measurements were made with standardised Ostwald viscometer tubes at 25°. For measurements on culture media, care was taken to obtain representative samples. For measurements on purified dextran, aqueous solutions were used after filtration through sintered glass funnel to remove traces of lint.

pH. Measurements were made with a Cambridge pH meter.

Chromatography. Whatman paper No. 1 was used for paper chromatography. Butanol–acetic acid–water (25.5:6.5: 10.5) was used as solvent. Spots were detected by spraying the paper with 1% silver nitrate in acetone followed by dipping in 5% ethanolic sodium hydroxide solution and then washing with 20% aqueous sodium thiosulphate solution.

Specific Rotation. Specific rotation was determined in 1N KOH with Schmidt–Haensch polarimeter.

Results and Discussion

The locally isolated strain of *Leuconostoc mesenteroides* produced 29 g purified dextran from 100 g sucrose in a culture medium containing (g/l) tryptone 10.0, yeast extract 5.0, sodium chloride 1.0 and dipotassium hydrogen phosphate 1.0 in 18 hr at 25–27° (Table 1). This composition of the media was selected on experimental basis. When sodium chloride and dipotassium hydrogen phosphate were excluded from the culture medium no growth of the bacteria was observed. Keeping the manner of inoculation, temperature and incubation time constant, experiments, where less than 10.0 and 5.0 g tryptone and yeast extract respectively in 1 litre culture medium were used, the yield of dextran decreased from 29 to 25% of sucrose. However, if the incubation was allowed to proceed for another 72 hr the yield of dextran again reached a maximum (29% sucrose). Probably due to lack of sufficient nutrients, the bacteria became inefficient and took more time to produce dextran from sucrose. The incubation time used previously, for the production of dextran has varied from 18 hr to 20 days.[3,4] Increase in the quantity of sucrose substrate by 50–100% while keeping other ingredient and manipulation constant also resulted in lower yield, (20% sucrose, Table 2).

The optimum temperature for incubation was found to be 25–27°. At a lower temperature (18°) no growth of the bacteria was observed, while at 37° a poor yield of only 10% of initial sucrose was obtained. At an elevated temperature of 45° bacteria ceased to grow completely (Table 3).

The production of dextran was followed by determining the pH and viscosity of the culture medium and isolating the dextran from the culture medium by precipitation (Table 1). The pH of the culture medium decreased from 8 to 4.5, the viscosity reached a maximum of 106.0 centipoise and the yield of the purified dextran isolated by precipitation (Table 1) was also maximum 29% of sucrose or 58% of the available glucose, after 18-hr incubation. It was observed that after 24 hr the viscosity of the culture medium began to decrease. The decrease in the viscosity of the culture medium after 24 hr is mainly due to autolysis of dextran caused by the enzymes present in the culture medium.[3] However, it is not known whether autolysis results in some selective structural changes in dextran or merely produces a random decrease in molecular size.

TABLE 1. CHANGE OF pH AND VISCOSITY OF THE CULTURE MEDIUM (100 g SUCROSE) AND THE YIELD OF DEXTRAN DURING INCUBATION AT 25°.

Incubation time (hr)	pH	Density	Absolute viscosity c.p. in (25°)	Yield of dextran
0	8	1.041	1.56	—
2	7	1.042	1.60	—
4	7	1.042	1.76	—
6	6	1.042	3.20	—
12	5	1.041	5.8	25.0
18	4.5	1.035	106.0	29.0
24	4.5	1.031	100.0	29.0

TABLE 2. EFFECT OF DIFFERENT CONCENTRATION OF SUCROSE ON THE PRODUCTION OF DEXTRAN IN 100-ml CULTURE MEDIUM.

Wt of sucrose (g)	Incubation time (hr)	Final pH of the culture medium	Yield of dextran
5	18	4.5	1.4
10	18	4.5	2.9
15	18	5.0	3.0
20	18	5.0	3.0

TABLE 3. EFFECT OF INCUBATION TEMPERATURE ON THE PRODUCTION OF DEXTRAN FROM 10 g.

Temparature in C°	Incubation time (hr)	Final pH of the culture medium	Yield of dextran (g)
18	18	7.0	Nil
25	18	4.5	2.9
27	18	4.5	2.9
37	18	6.0	1.0
45	18	7.0	Nil

TABLE 4. EFFECT OF DIFFERENT CONCENTRATION OF THE EXTRACTS OF RICE-POLISHING AND WHEAT-BRAN ON THE PRODUCTION OF DEXTRAN FROM 10.0 g SUCROSE.

Wt of rice-polishing (g)	Incubation time (hr)	Final pH of the culture medium	Yield of dextran (g)
Rice-polishing			
0.5	18	6.0	1.0
1.0	18	4.5	2.9
3.0	18	4.5	2.9
Wheat-bran			
0.25	18	5.0	1.2
0.50	18	4.5	2.9
1.0	18	4.5	2.9

Properties of Purified Dextran

Moisture 5.8%; dextran, 94.0%; reducing sugar as glucose, 0.06%; ketoses (fructose), nil.

Composition. On complete acid hydrolysis yielded glucose (identified by paper chromatography and specific rotation).

Solubility. Readily soluble in water, but solution appeared opalescent.

Specific Rotation. + 198 (C=0.5 in 1N KOH)

Relative Viscosity. 1.076, 1.412, 1.708 and 2.267 (C= 0.2, 0.4, 0.6 and 1.0 at 31°).

Rice-polishing and wheat-bran, abundantly available in this country, contain adequate quantities of protein and vitamins. Their extracts were prepared for incorporation in the culture medium in place of tryptone and yeast extract which are imported items. As given in Table 4 the extract of 1 g rice-polishing or 0.5 g of wheat-bran were found to be sufficient for both the growth of the bacteria and the production of dextran from 10 g sucrose in 100 ml culture medium. Excess amount of the extract of rice-polishing (3 g) or wheat-bran (1 g) do not show any adverse effect on the yield of the purified dextran as has been observed when the quantity of the substrate was increased to 15 and 20 g in 100-ml culture medium (Table 3). Fresh rice-polishing and wheat-bran have invariably been used, since their nutrient value was lost on keeping. The properties of the purified dextran as given below compared well with that obtained when yeast extract and tryptone were used in the culture medium.

Properties of Purified Dextran

Moisture, 6.0%; dextran, 93.5%; reducing sugars as glucose, 0.08%; ketoses (fructose), nil.

Composition. On complete acid hydrolysis yielded glucose.

Solubility. Readily soluble in water but solution appeared opalescent.

Specific Rotation. +199 (C= 0.5 in 1N KOH)

Relative Viscosity. 1.076, 1.412, 1.708 and 2.267 (C=0.2, 0.4, 0.6 and 1.0 at 31°).

Acknowledgement. The authors are thankful to Mr. S. Ashfaq Hussain for useful help in conducting the experiments.

Reference

1. T.H. Evans and H. Hibbert, *Advances in Carbohydrate Chemistry*, edited by W.W. Pigman and M.L. Wolfrom (Academic, New York, 1946), vol. II, p. 203 and vol. XV, p. 341 (1960).
2. J.Y. Sugg and E.J. Hehre, J. Immunol., **43,** 119 (1942).
3. A. Jeanes, C.A. Wilham, and J.C. Miers, J. Biol. Chem., **176**, 603 (1948).
4. A. Jeanes and C.A. Wilham, J. Am. Chem. Soc., **72,** 2655 (1950).
5. S.A.H. Zaidi, Rabia Zuberi and Jameela Jafri, Pakistan Patent, 122632 (April 1972).

6. *Bergey's Mannual of Determinative Bacteriology*, edited by R.S. Bred, E.G.D. Murrey and N.R. Smith (Bailliere, Tindall and Cox, London, 1957), pp. 531.
7. D. Glick, *Methods of Biochemical Analysis* (Interscience, New York, 1955), vol. II, pp.325.
8. P.B. Hawk, B.L. Oser, W.H. Summerson, *Practical physiological Chemistry* (McGraw, New York, 1954), pp. 568.