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Production of dextran by *Leuconostoc mesenteroides* TISTR 053 in fed batch fermentation

Piyarat Nuwan[1], Phakphimol Piwpan[1], Adisak Jaturapiree[2] and Phimchanok Jaturapiree[1,]*

[1]Department of Biotechnology, Faculty of Engineering and Industrial Technology, Silpakorn University, Muang, Nakhon Pathom, Thailand 73000.
[2]Research Unit of Agriculture Residues and Biomaterials, Division of Chemistry, Faculty of Science and Technology, Nakhon Pathom Rajabhat University, Muang, Nakhon Pathom, Thailand 73000.
[*]Corresponding author: phimchanok@hotmail.com

Abstract

Dextran is a natural homopolysaccharide composed of α(1,6)-linkages in their major chain and is synthesized by dextransucrase of the microbial cell in the presence of sucrose as a substrate. The production of dextran by sucrose fermentation using *Leuconostoc mesenteroides* TISTR 053 was carried out in a Biostat B plus fermenter with total working volume of 3 liter. The effects of sucrose concentrations and modes of operation (batch and fed batch) were studied. The maximum dextran production obtained after 24 hours of incubation at 37 ^{o}C with 20% sucrose in batch fermentation. The fed batch fermentation promoted the dextran productivity. The structure of dextran was determined and confirmed by FTIR and NMR.

Keywords : *dextran, fed batch fermentation, Leuconostoc, production*

1. Introduction

Dextrans, $(C_6H_{10}O_5)_n$ are now defined as natural homopolysaccharides of glucose with predominant α(1,6)-linkages in the linear chain with three different kinds of branched linkages [α(1,2), α(1,3), α(1,4)] depened on the extracellular enzyme, dextransucrase. This enzyme is produced by several organisms such as lactic acid bacteria *viz*., *Leuconostoc*, *Lactobacillus*, *Streptococcus* and *Weisella* in the presence of sucrose as a substrate [1-4]. Dextran is widely applied in food, textile, pharmaceutical, fine chemicals, cosmetics, agricultural and oil drilling industries due to their wide diversity in structure and physical properties. In food industry, dextrans are used as emulsifying, viscosifying, texturizing, thickening and gelling agents in food formulations. It is used as drug especially as a blood plasma volume expander, iron carrier or anticoagulant in pharmacy. A chromatographic media, cross-linked dextran, e.g. Sephadex, is widely used in research and industry for separation and purification of protein [5-8]. Several researchers have optimized fermentation conditions for maximum dextran production. It has been reported earlier that the production and the properties of dextran are influenced by bacterial strain, culture medium, process variables (pH,

temperature, agitation speed and time of fermentation) and modes of operation (batch, fed batch and immobilized cell) [9-11]. Batch fermentation is available in a commercial production of dextran in a sucrose-rich media. When high sucrose concentration in the medium is used to produce dextran, the polymer concentration becomes high and causes the viscosity of the broth. The process of controlling, separation, handling, and pumping become more difficult. The fed batch operation may be better than the conventional batch operation, especially with the broth of high viscosity. Therefore, decreasing viscosity of the broth by the addition of a water-based feed, a possible increase in dextran production is expected [12]. Another advantage of this process is the higher productivity due to the decreasing of fermentation time. The goal of this work was to study the effects of sucrose concentrations and operation modes (batch and fed batch) on the growth of *Leuconostoc mesenteroides* TISTR 053 and the production of dextran. The structure of dextran was also analyzed and confirmed by FTIR and NMR.

2. Materials and Methods

2.1 Microorganism

The microorganism for the production of dextran in this study was *Leuconostoc mesenteroides* TISTR 053, from the Thailand Institute of Scientific and Technological Research Culture Collection. The strain was maintained in Dextran Medium Agar (DMA) at 4 °C. The compositions of the DMA were: 100 g/L sucrose, 10 g/L Typtone, 1 g/L yeast extract, 2.5 g/L K_2HPO_4 and 10 g/L agar [9]. The cultures were maintained by sub-culturing every week and the plates were incubated at 30 °C for 24 hours. For the stock culture, the strain was stored in DMA supplemented with 80% glycerol at -80 °C.

2.2 Inoculum preparation

Leuconostoc mesenteroides TISTR 053 was cultured in 250 mL Erlenmeyer flasks, containing 50 mL of the Dextran Medium Broth 1 (DMB1). This medium was autoclaved at 121°C for 15 minutes prior to inoculation. The strain was grown in DMB1 by incubating at 30 °C for 24 hours on a rotary shaker at 200 rpm.

2.3 Fermentation Medium

To study the production of dextran from *L. mesenteroides* TISTR 053, the Dextran Medium Broth 2 (DMB2) was used. One litre of DMB2 was prepared as following sucrose (100, 150 or 200 g/L), Becto-peptne (5 g/L), yeast extract (5 g/L), K_2HPO_4 (15 g/l), $MnSO_4 \cdot H_2O$ (0.01 g/L), NaCl 0.01 g/L0 and $CaCl_2 \cdot 2H_2O$ (0.05 g/L) [9]. The pH of the medium was adjusted to 7.0.

2.4 Bioreactor

A 5-L Biostat B. plus bioreactor (Sartorius) installed with a micro DCU-Twin and MCU-200 controllers with a 3-L working volume was used for the production of dextran in the aerobic fermentations of *L. mesenteroides* TISTR 053. The reactor was controlled at 30 °C by an external cooling bath with constant agitation of 150 rpm and aerated with sterile air at an aeration rate of 1 VVM. The pH was measured on-line using a pH probe connected to the micro DCU system and controlled at 7.0 with 1M NaOH or 1 M HCl.

2.5 Fermentation Modes

2.5.1 Batch Fermentation

The effect of initial sucrose concentrations on the dextran production was studied in batch operation. The fermentation was carried out in a 5-L bioreactor with a working volume of 3-L. The bioreactor with 2.7 L of DMB2 medium was sterilized at 121°C for 15 min. After cooling to appropriate temperature, it was inoculated with the 300 mL inoculums. The temperature, pH, agitation speed and aeration rate were controlled as describe above. Samples were withdrawn aseptically every 2 hours for the dermination of total sugar, dextran and growth. Broth samples collected at the end of the fermentation were used to study dextran properties.

2.5.2 Fed Batch Fermentation

When total sugar in culture decreased less than 10 g/L, the 500 mL of the concentrated sucrose (500 g/L) was fed continuously into the bioreactor with a peristaltic pump. The impeller speed was set at 200 rpm and 30ºC, with the air flow rate of 1 vvm for all runs.

2.6 Analytical Measurements

Samples were withdrawn from the bioreactor every 2 hours and centrifuged at 4,000 rpm for 10 min. The precipitate was afterwards used for cell growth determination by spectrophotometry, while the supernatant was used for total sugar and dextran determination by the below procedure

2.6.1 Assay of Cell Growth

Cell growth was measured by spectrophotometer at 600 nm. The cell dry weight was obtained from a calibration curve of dry weight against O.D. (600 nm). The calibration curve was linear up to the cell dry weight (X) of 100 mg/L, corresponding to the cell turbidity (absorbance) of 0.394 at 600 nm.

2.6.2 Assay of Sugar

The concentration of total sugar was determined by phenol-H_2SO_4 method [13].

2.6.3 Assay of Dextran

Dextran was precipitated from the supernatant using chilled 95% ethanol (ratio 1:1), and stored overnight at 4 °C. The precipitate was collected by centrifugation at 4,000 rpm at 4 °C for 20 min and dried at 30 °C over $CaCl_2$ in desicator until constant weight [9]. The dextran yield was determined by weighing the polysaccharide mass. All experiments were performed in triplicates and average values were reported.

2.7 Characterization of Dextran

2.7.1 Fourier transform infrared (FTIR)

The FTIR spectrum of the produced dextran from *L. mesenteroides* TISTR 053 in DMB2 was analyzed by spectrophotometer at 500-4000 (cm^{-1}). Prior to FTIR spectronic analysis, the sample was mixed with KBr (spectroscopic grade), milled and pressed into a 2 mm pellet.

2.7.2 Nuclear magnetic resonance (NMR)

For the determination of the glycosidic linkage types, a nuclear magnetic resonance spectroscopic technique (^{1}H NMR) was used. The dextran was dissolved in D_2O solution and the spectrum was recorded using a NMR spectrophotometer.

3. Results and Dicussion

Leuconostoc mesenteroides TISTR 053 showed highly viscous slimy and shiny colonies on sucrose containing medium (Figure 1). The similar observation was

reported in case of *L. mesenteroides* CMG713. When the strain was streaked on the agar plate containing sucrose, the colonies presented slimy and shiny. A typical growth phase of *L. mesenteroides* TISTR 053 in batch fermentation with 150 g/L sucrose in the medium could be presented in Figure 2, including the following phases: lag phase (0 - 4 h.), exponential growth phase (4-16 h.) and stationary phase (16-24 h.). During lag phase, a slight increase in cell concentration occurred because of the bacteria adapted themselves to growth conditions. The strain grew exponentially and reached the maximum biomass and dextran concentration of 37 and 35 g/L, respectively. These concentration levels of dextran were kept until the end of fermentation, coinciding with a decreasing in the concentration of sucrose. This character occurred because of the cells consumed sucrose for cell growth and dextran production. After 16 hours of fermentation, the growth rate was found to slow down as the result of sucrose depletion.

Figure 1. Slimy and shiny colonies of L. mesenteroides TISTR 053 on medium containing sucrose.

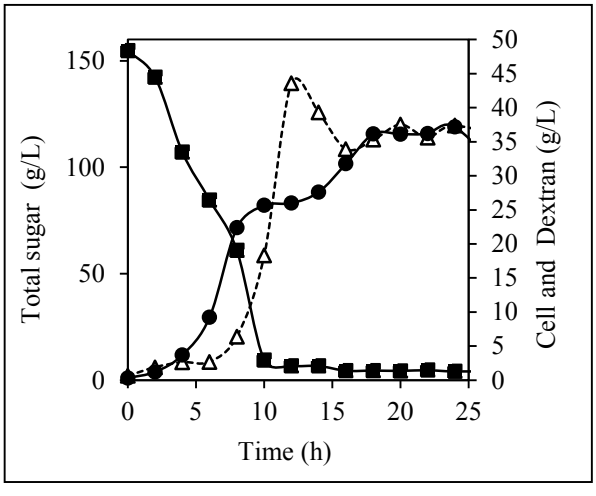

Figure 2. A typical growth curve and dextran production in batch culture by L. mesenteroides TISTR 053 in medium containing 150 g/L sucrose: cell concentration (•), total sugar (■), dextran concentration (Δ)

3.1 Effects of initial sucrose concentrations

To study the effect of initial sucrose concentrations, the range of sucrose concentrations from 100 to 200 g/L were used. Increasing in the concentration of initial sucrose increased the maximum dextran production from 30 to 45 g/L. This may be due to the more activity of dextransucrase enhanced by the higher levels of sucrose in medium as the same result reported by Kim et. al. [14]. The effects of sucrose concentration on the yield of dextran by a mutant strain of *L. mesenteroides* B512 FMCM. Increasing the sucrose concentration enhanced in the yield of dextran was also reported by Tsuchiya et. al. [15]. This trend agreed with the report by Sarwat et. al. [9] and Vedyashkina et. al. [11]. From the growth curves, the kinetic parameters, maximum specific growth rate (μ_{max}), dextran concentration and dextran productivity (Q_p) were calculated and presented in Table 1. It indicated that the higher concentrations of sucrose led to the higher dextran concentration and productivity.

Table 1. The kinetic parameters of dextran production by L. mesenteroides TISTR 053 in batch fermentation.

Initial sucrose concentration (g/L)	maximum specific growth rate (h^{-1})	dextran concentration (g/L)	dextran productivity (g/L·h)
100	0.29	30	1.25
150	0.45	35	1.46
200	0.41	45	1.88

3.2 Effects of operation modes

In batch fermentation, the higher concentration of sucrose gave the more viscosity of the fermentation broth. The mass and oxygen transfer rate were poor. Therefore, decreasing of the viscosity broth by addition of a water-based feed, possibly increased the dextran production. Our study indicated that fed batch fermentation gave a better cell and dextran concentration and dextran productivity. The feeding of the concentrated sucrose solution (500 g/L) 500mL was initiated when the most of the substrate had been consumed and the growth of bacteria was at the end of exponential phase (Figure 3). It was observed that sucrose was depleted after 10 hours of fermentation time. The fed batch mode was indicated to produce the dextran concentration up to the maximum 56 g/L. The kinetic parameters of dextran production by batch and fed batch were calculated and compared in Table 2. The results of this study indicated that the fed batch fermentation promoted the dextran concentration and productivity.

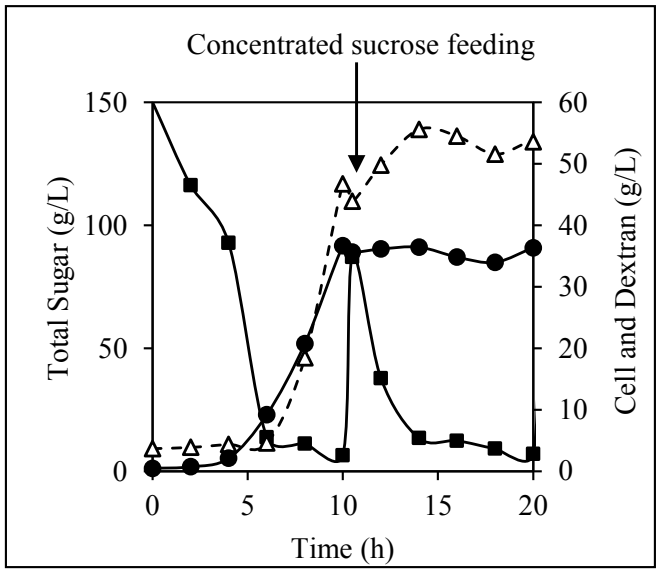

Figure 3. Fed batch fermentation of L. mesenteroides TISTR 053 during dextran production with initial sucrose concentration of 150 g/L: cell concentration (•), total sugar (■), dextran concentration (Δ)

Table 2. The kinetics parameters of the dextran production by L. mesenteroides TISTR 053 in batch and fed batch fermentation.

Fermentation modes	dextran concentration (g/L)	dextran productivity (g/L·h)
Batch	35	1.46
Fed batch	55	2.75

3.3 FTIR spectrometry of Dextran

The FTIR was used to investigate the nature of the functional groups, monomeric units and linkages presented in the dextran. In current study, the purified dextran produced by *L. mesenteroides* TISTR 053 showed the band in the region of 3438 cm^{-1}, 2924 cm^{-1}, 1637 cm^{-1}, and 1018 cm^{-1} which represented the hydroxyl stretching vibration of a polysaccharide, C-H stretching vibration, carboxyl group, vibration of C-O bond at C-4 position of D-glucose and the great chain flexibility around the α-(1,6) glycosidic bond, respectively (Figure 4). These results were in accordance with the reports from many researchers [16-19]. The comparative FTIR spectrum in different studies are shown in Table 3.

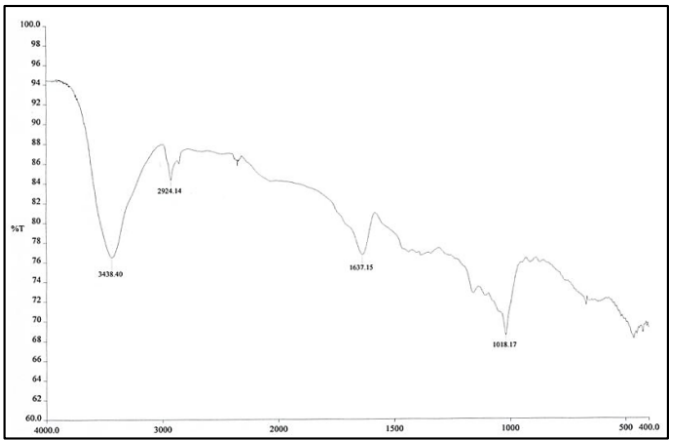

Figure 4. The FTIR spectrum of the dextran produced from L. mesenteroides TISTR 053.

Table 3. The FTIR spectrum comparison of the dextran in different studies.

Peak assignment	Peak position (cm^{-1})			
	1*	2*	3*	4*
(OH) stretching	3438	3400	3434	
(CH) vibration	2924	2930	2928	2935
(CO) stertching	1637	1639	1639	1641
α(1,6) glycosidic bonds	1018	1020	1020	1019

* *L. mesenteroides* TISTR 053 [This study]
2* *Strongylocentrotus* [17]
3* *L. mesenteroides* B640 [18]
4* *W. cibaria* CMGDEX3 [19]

^{1}H-NMR spectrometry of Dextran

The ^{1}H-NMR spectra of dextran produced from *L. mesenteroides* TISTR 053 was shown in Figure 5. The anomeric proton signal showed six spectral resonances at 4.99, 3.59, 3.74, 3.54, 3.92 and 4.00 ppm corresponding to H-1, H-2, H-3, H-4, H-5 and H-6, respectively. The distribution signals in the ^{1}H- NMR spectra resonance between 3 and 6 ppm for different dextrans were assigned as described earlier (Table 4). The anomeric resonance peak at 4.99 ppm was corresponded to the H-1 of a typical α(1,6) glucosyl linkage of dextran. Seymour reported that the resonance peak of the dextran from *L. mesenteroides* NRRL B-1355 at 5.3 ppm in ^{1}H-NMR spectrum was assigned for the branched linkages [20]. Hence, the absence of peak near 5.3 ppm of the dextran from *L. mesenteroides* TISTR 053, confirmed its linear nature.

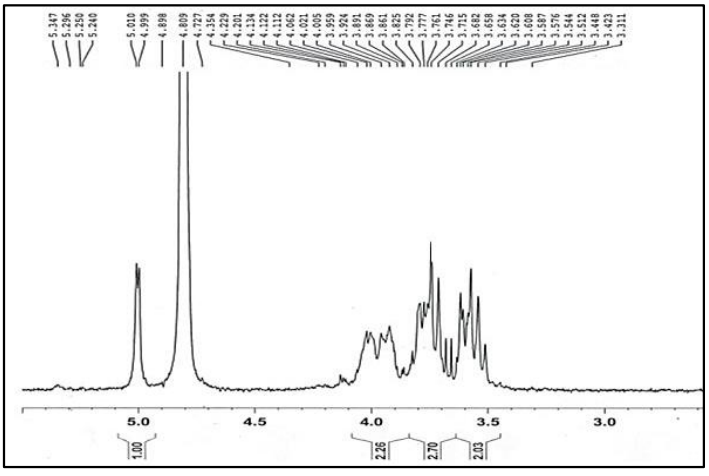

Figure 5. ^{1}H NMR sprectrum of dextran produced from L. mesenteroides TISTR 053

Table 4. The assignments of ^{1}H NMR chemical shift values of different dextrans

Strain	H-1	H-2	H-3	H-4	H-5	H-6
1*	4.99	3.59	3.74	3.54	3.92	4.00
2*	4.98	3.58	3.73	3.54	3.92	3.99
3*	4.98	3.58	3.73	3.54	3.93	3.99

1* *L. mesenteroides* TISTR 053 [This study]
2* *L. mesenteroides* B640 [18]
3* *W. cibaria* CMGDEX3[19]

4. Conclusions

In this work, the dextran synthesized by L. mesenteroides TISTR 053 in medium containing sucrose with fed batch fermentation by feeding the concentrated sucrose solution was successful to enhance the production of dextran. The maximum dextran concentration and productivity were 55 g/L and 2.75 g/L.h, respectively. The structure of dextran has a linear α(1,6)-linkage which was determined and confirmed by FTIR and NMR.

5. Acknowledgement

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