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Co-Production of Dextran and Mannitol by *Leuconostoc mesenteroides*

YOO, SUN KYUN[1]*, DOMAN KIM[1,2], AND DONAL F. DAY[3]

[1]*Engineering Research Institute,* [2]*Division of Chemical Engineering and The Research Institute for Catalysis, Chonnam National University, Kwangju 500-757, Korea*
[3]*Audubon Sugar Institute, Louisiana State University, Agricultural Center, South Stadium Dr., Baton Rouge, LA 70803, U.S.A.*

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Abstract To optimize co-production of dextran and mannitol from sucrose by *Leuconostoc mesenterides* ATCC 13146, a batch culture fermentation was conducted by using various concentrations of sucrose and initial culture pHs. The production of dextran and mannitol showed a growth-associated pattern. The highest yields of both dextran and mannitol were obtained at pH 6.0 and 10% (w/v) sucrose. They could be easily separated by using alcohol fractionation. Maximum yields of dextran and mannitol were 0.45 and 0.35 of the consumed sucrose, respectively. Overall productivities of dextran and mannitol were 1.47 and 0.37 g/l/h, respectively.

Key words: Functional foods, fermentation, optimization, dextran, mannitol, *Leuconostoc mesenteroides*

Consumer demand for functional foods such as GRAS (generally recognized as safe) exopolysaccharides and mannitol is rapidly increasing [1, 4]. Dextran is a generic term for a bacterial exopolysaccharide synthesized from sucrose and composed of chains of D-glucose units connected by α-1,6-linkages by using dextransucrases [15]. Dextran can be branched out by α-1,2, α-1,3, or α-1,4-linkages, to varying degrees, depending on the strain of *Leuconostoc* sp. [15]. *L. mesenteroides* dextransucrases can only be produced by sucrose induction, with the exception of the constitutive mutants which are produced from *Leuconostoc* sp. [6]. In addition to catalyzing the synthesis of dextrans from sucrose, dextransucrase can also catalyze the transfer of glucose to sucrose to other carbohydrates which are present or have been added to the reaction digest [7-9, 12]. Dextran could be used as vicosifying, stabilizing, emulsifying, gelling, bulking, dietary fiber, and water holding agents [14]. When grown on sucrose, *Leuconostoc mesenteroides* ATCC 13146 produces two dextransucrases which synthesize two branched dextrans: soluble (S) type and less soluble (L) type [6]. Soluble dextran (precipitated by 45% ethanol) contains 50% of α-D-(1-6) glucose linkages and 50% of α-(1-3) branch linkages. Less soluble dextran (precipitated by 39% alcohol) is made up of 87% of α-D-(1-6) glucose linkages and 13% of α-D-(1-4) branch linkages [15]. Fructose is liberated from sucrose and is partially metabolized by bacteria [2]. An ability for heterolactic fermentative bacteria to reduce fructose to mannitol has been well known [11. 16. 21]. We have found that *L. mesenteroides* ATCC 13146 quantitatively converted fructose to mannitol by a mannitol dehydrogenase. Mannitol is used as a food ingredient, anticaking, stabilizing, thickening, and texturizing agents, and as a medical treatment for reducing plasma hydrogen peroxide in patients undergoing coronary artery bypass graft surgery [20, 22]. Since the early literature contained few reports of the co-production of dextran and mannitol, we have conducted a batch culture fermentation to produce dextran and mannitol simultaneously. In this study, we report how initial medium pH and sucrose concentrations affect the yield of dextran and mannitol. The main purpose of this study was to optimize microbial co-production of dextran and mannitol by utilizing a simple dextran production process.

L. mesenteroides ATCC 13146 was purchased from American Type Culture Collection (Rockville, MD, U.S.A.) and maintained on the agar slant medium with the composition of 3.0 g K_2HPO_4, 0.01 g $FeSO_4 \cdot H_2O$, 0.01 g $MnSO_4 \cdot 7H_2O$, 0.01 g NaCl, 0.05 g $CaCl_2$, 0.5 g yeast extract, 15 g agar, and 30 g sucrose per liter deionized water. The medium pH was adjusted to 6.0 prior to sterilization. Working cultures for inocula were prepared by transferring a loopful of colony from the agar slant to culture tubes (20×125 mm) containing 10 ml of liquid medium (excluded agar from the agar slant medium) and incubating the culture tubes at 25°C and 200 rpm for 24 h. For determining the pH optimum, cultures were prepared in tubes (20×150 mm) containing 10 ml of liquid medium and 2% sucrose. The

*Corresponding author
Phone: 82-62-530-1992; Fax: 82-62-530-1973;
E-mail: skyoo@chonnam.chonnam.ac.kr

inoculum was 2% (v/v) of liquid medium. The medium was adjusted to a pH ranging from 5.0 to 7.5 prior to sterilization. The culture tubes were incubated in a rotary shaker (NBS model G25-KC, Edison, NJ, U.S.A.) at conditions of 28°C and 200 rpm for 10 h. For determining the optimum sucrose concentration, various concentrations of sucrose ranging from 20 to 400 g/l were added to culture tubes (20× 150 mm) containing 10 ml of liquid medium. The medium pH was adjusted to 6.0. Culture tubes were incubated under the previously established conditions. To investigate the growth and product formation kinetics, batch culture fermentation was performed in a BioFlo II fermenter (NBS) with a working volume of 1.2 l by using 18% (w/v) sucrose under optimum pH condition. The inoculum was 2% (v/v) of the working volume. Fermentation conditions were 28°C, 100 rpm of agitation, and 1 vvm of aeration. The fermentation process continued until the sucrose was consumed completely (50 h).

Bacterial cells were removed by centrifugation at 4°C and 10,400 ×g for 20 min (Dupont Sorvall RC5C, Newtown, CT, U.S.A.). To precipitate dextran, 95% (v/v) of alcohol was added with slow stirring to the supernatant until the volume of ethanol reached two thirds of that supernatant. The solution was allowed to stand for 2 h and centrifuged at 3,000 ×g for 20 min. The precipitate (dextran) was washed twice with deionized water. Then, the dextran solution (about 10%) was lyophilized. To recover mannitol, the dextran-depleted solution was concentrated to 50° brix by using a rotary vacuum evaporator (Rotavapor R110, Buchi, Switzerland). The concentrated solution was stored at −20°C to crystallize mannitol. The crude crystallized mannitol was filtered, washed with 95% (v/v) alcohol, and then lyophilized. The lyophilized dextran was dissolved with deionized water to make a 10% (w/v) dextran solution. To fractionate the L-dextran, alcohol was added to the dextran solution until the alcohol concentration reached 39% (w/v), and the mixture was then centrifuged under the previously established condition. To recover S-dextran, alcohol was continuously added to the remaining solution until the alcohol volume reached 45% (v/v). The dextran size was determined by a gel permeation chromatography using an Ultrahydrogel Linear column (Millipore, MA, U.S.A.) and a differential refractometer (Millipore, MA, U.S.A.). The eluent was deionized water. The rate of elution was 1.0 ml/min. The column temperature was 20°C. Dextrans, T 5 (average molecular weight 5,000), 10, 40, 70, 150, 500, and 2,000 (Pharmacia, Piscataway, NJ, U.S.A.) were used as standards.

Bacterial growth was estimated by a modification of the method developed by Otts and Day [13]. To determine concentration changes of sugars during fermentation, the cell free supernatant of samples was adjusted to pH 7.0 and a known amount of sorbitol was added as an internal standard. The sample was filtered through a 0.22 μm membrane. Sugar, except dextran, were determined by

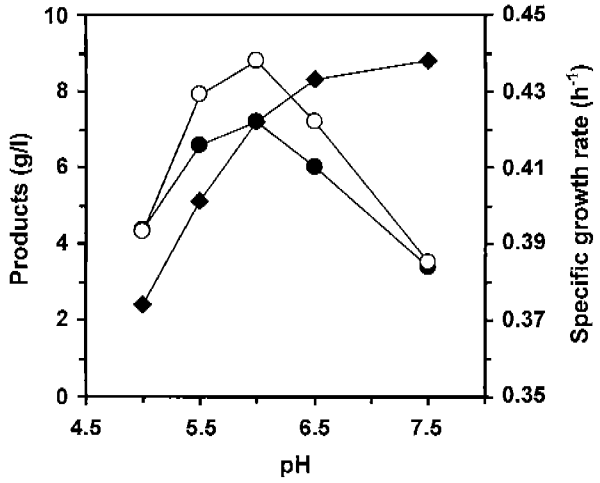

Fig. 1. Dextran and mannitol production by *Leuconostoc mesenteroides* ATCC 13146 at various initial culture pHs.
Fermentation was conducted in 2% sucrose, at 28°C and 200 rpm for 10 h. ● dextran, ○ mannitol, and ◆ specific growth rate.

HPLC (Waters Millipore, Milford, MA, U.S.A.) using an ion-exchange/size-exclusion column (Sugarpak, Millipore Corporation, Milford, MA, U.S.A.) under the following conditions: temperature, 90°C; the eluent, deionized water; the eluent rate, 0.5 ml/min. The detector was a model 410 Differential Refractometer (Millipore Corporation, Milford, MA, U.S.A.). The quantification of dextran was assayed by the phenol-sulfuric acid methods [3].

The growth and carbohydrate production of *L. mesenteroides* ATCC 13146 varied depending on the initial culture pH. Figure 1 shows the final amount of dextran and mannitol in addition to the maximum specific growth rate which were calculated from fermentation kinetics at various initial culture pHs. The culture pH of the optimum growth (pH 7.5) did not correspond to that of mannitol or dextran production (pH 6.0). Dextran and mannitol production showed a similar trend with increased initial culture pH from 5.0 to 7.5, i.e. they increased from pH 5.0 to 6.0 and declined to pH 7.5. However, the specific growth rate rose constantly with increasing culture pH. This demonstrates that culture pH influences differently for bacterial growth and for the production of dextran and mannitol. Tsuchiya *et al.* [18] demonstrated that the maximum dextransucrase production was reached at pH 6.7. However, the maximum dextran production was observed at pH 6.1. The yield ($Y_{d,m/s}$) and specific yield ($y_{d,m/x}$) of dextran and mannitol also varied depending on initial culture pHs (Table 1). Both values were increased from pH 5.0 to 6.0 and declined to pH 7.0. The optimum culture pH for the production of dextran is consistent with previous results. The maximum production of dextran with *L. mesenteroides* was obtained at the pH range of 5.5 to 6.1 [10, 18]. This is because the proton motive force, which regulates extracellular dextransucrase production, is relatively constant in the pH

Table 1. The effect of pH and sucrose concentration on the production of dextran and mannitol by *Leuconostoc mesenteroides* ATCC 13146.

	X	$Y_{d/s}$	$Y_{m/s}$	$y_{d/x}$	$y_{m/x}$
pH					
5.0	0.96	0.28	0.28	4.48	4.52
5.5	1.08	0.39	0.33	7.31	6.11
6.0	1.01	0.44	0.36	8.22	6.73
6.5	1.27	0.36	0.30	5.67	4.72
7.0	1.47	0.24	0.23	2.38	2.31
Sucrose (g /l)					
20	1.28	0.35	0.30	5.61	0.24
100	1.27	0.45	0.35	14.1	11.00
200	1.21	0.15	0.23	8.01	12.30
300	0.92	0.02	0.18	1.95	17.06
500	0.37	0.00	0.11	0.00	41.35

X, Biomass (g/l). $Y_{d/s}$, dextran/sucrose consumed (g/g). $Y_{m/s}$, mannitol/sucrose consumed (g/g). $y_{d/x}$, dextran/biomass (g/g). $y_{m/x}$, mannitol/biomass (g/g).

range from 5.5 to 7.0 [13]. The growth of *L. mesenteroides* ATCC 13146 and the production of dextran and mannitol also varied depending on the sucrose concentration (Fig. 2). Growth inhibition appeared to have increased as the sucrose concentrations increased from 20 to 400 g/l. The growth rate decreased linearly from 0.73 to 0.21/h as the sucrose concentration increased. However, dextran production increased up to 100 g/l sucrose concentration, but rapidly decreased to 400 g/l sucrose, where no dextran was detected. Mannitol production steadily rose with increasing sucrose concentration. The yield and specific yield of dextran and mannitol are shown at Table 1. The highest yield of dextran and mannitol was found at 100 g/l sucrose. Interestingly, the highest specific yield of dextran was found at 100 g/l sucrose, where its value was $y_{d/x}$=14.1, whereas that of mannitol was found at 500 g/l sucrose, where its value was $y_{m/x}$=41.35. Our results corresponded with previous results showing that the sucrose concentration was greater than 200 mM (about 69 g/l) of substrate when inhibition occurred [17], and in the concentration higher than 2 M (700 g/l) sucrose, no higher molecular weight dextran was produced [19]. A less than 2% of dextran yield was shown at more than 300 g/l sucrose concentration. But mannitol was still produced with the yield of about 20%. Bacterial growth and sucrose fermentation kinetics were investigated until the sucrose was completely utilized (Fig. 3). The concentration of sucrose decreased rapidly when bacterial growth reached a stationary phase (20 h) and continued to decrease slowly to 50 h fermentation (Fig. 3A). As sucrose became limiting, regardless of the presence of fructose and mannitol, bacterial growth was suppressed. What this implies is that bacterial growth has links to dextran production, but is not associated with mannitol production. At the log growth phase, the specific growth rate was 0.54/h and sucrose utilization was about 5.8 g/l/h. Dextran production continued until the sucrose was completely consumed (Fig. 3B). During fermentation, fructose was accumulated until the early stationery growth phase and decreased rapidly during the stationary phase. Fructose is converted to mannitol by mannitol dehydrogenase [2]. Interestingly enough, the

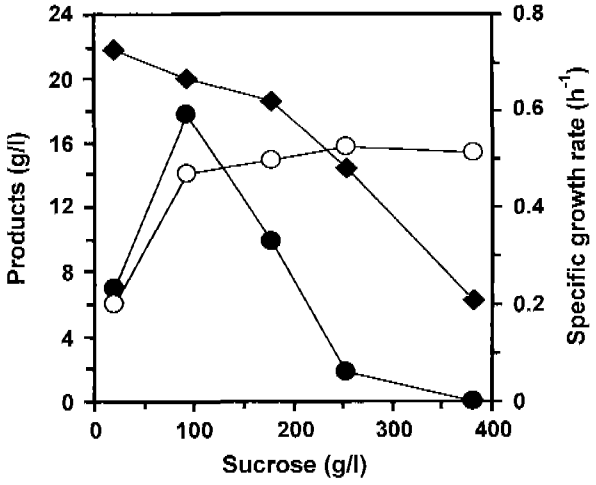

Fig. 2. Dextran and mannitol production by *Leuconostoc mesenteroides* ATCC 13146 at various sucrose concentrations.
Fermentation was conducted at 28°C, pH 6.0, and 200 rpm for 10 h. ● dextran, ○ mannitol, and ◆ specific growth rate.

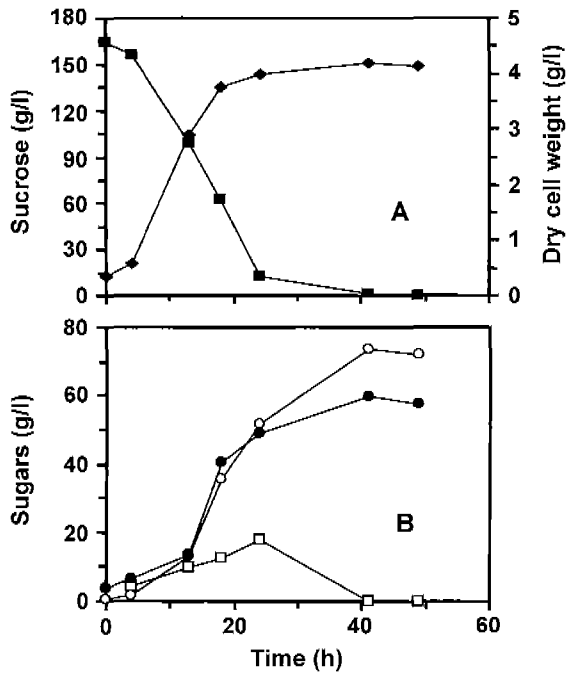

Fig. 3. The kinetics of dextran and mannitol production by *Leuconostoc mesenteroides* ATCC 13146 in a bioreactor.
Fermentation was conducted in 18% sucrose and at 28°C, pH 6.0, and 100 rpm. Aeration was 1 vvm. A: ■ sucrose and ◆ bacterial growth; B: ● dextran, ○ mannitol, and □ fructose.

conversion of fructose to mannitol started when sucrose utilization started, and mannitol production continued until no fructose remained. Maximum yields of dextran and mannitol were observed at the same fermentation time (41 h): the values were $Y_{d/s}$=0.38 and $Y_{m/s}$=0.45, respectively. The overall productivity of dextran and mannitol were 1.47 and 0.37 g/l/h, respectively. S- and L-dextrans were separated by ethanol fractionation; less soluble (L) dextran in 39% (v/v) alcohol, soluble (S) dextran in 45% (v/v) alcohol, and total dextran in 50% (v/v) ethanol. L-dextran was gummy and brittle in texture. It did not dissolve in water at room temperature (23°C), but dissolved after 10 min in boiling water (dextran, 2%) in which the solution was clear. S-Dextran was sticky and dissolved at the room temperature. The solution was hazy. The composition of dextran was 25.5% soluble and 74.5% less soluble dextran. The range of molecular weights of the dextrans were determined by using gel permeation chromatography (data not shown). L- Dextran (prepared with 39% alcohol, v/v) was eluted between T 500 and T 2000 dextrans and S-dextran was eluted between T 70 and T 150 dextrans. In the present study, we have shown the production of two different sizes of dextrans and mannitol from a simple batch fermentation with *L. mesenteroides* ATCC 13146 by using sucrose and by easily separating these products using conventional processes.

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