BIODIVERSITAS
Volume 23, Number 2, February 2022
Pages: 1154-1158

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d230260

Short Communication: Dextran production using *Leuconostoc mesenteroides* strains isolated from *Borassus flabellifer* sap

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Manuscript received: 15 December 2021. Revision accepted: 29 January 2022

Abstract. *Ma'unatin A, Harijono, Zubaidah E, Rifa'i M. 2022. Short Communication: Dextran production using Leuconostoc mesenteroides* strains isolated from *Borassus flabellifer sap. Biodiversitas 23: 1154-1158.* Exopolysaccharide (EPS) is a carbohydrate polymer secreted by several bacteria in their growth period, including Lactic Acid Bacteria (LAB). Dextran is a type of EPS produced by *Leuconostoc mesenteroides* and is used widely in the food and pharmaceutical fields. Therefore, this study aimed to evaluate the potency of two strains of *L. mesenteroides* isolated from *Borassus flabellifer* L. (lontar) sap, namely *L. mesenteroides* N5 and N7 for dextran production using the MRS broth medium supplemented with sucrose at varying concentrations of 5, 10, and 15% (w/v). The results showed that *L. mesenteroides* N5 produced dextran of 14.53 g/L in medium with 15% sucrose, while *L. mesenteroides* N7 yielded 13.16 g/L at 10% sucrose. The characteristics of selected dextran showed that it contains 89.93% total carbohydrate, 2.33% protein and has a surface structure with tight pore for dextran N5 while the dextran N7 contains 87.84% total carbohydrates, 2.22% protein and has looser porous surface structure.

Keywords: Dextran, exopolysaccharide, *Leuconostoc mesenteroides*, sucrose

INTRODUCTION

The growing benefits of Exopolysaccharide (EPS) cause an increase in the exploration of bacteria as producers of EPS. One of the important things is that these bacteria must be non-pathogenic to ensure the safety of their metabolite products. LAB is a group of Generally Regarded as Safe (GARS) bacteria so that the EPS produced is a safe biopolymer (Feng et al. 2018). EPS plays a psychological function in the interactions between bacterial cells, adhesion, and protecting cells against the extreme environment were bacteria grow (Abid et al. 2017).

EPS is classified into 2 groups: homopolysaccharides (HoPS) and heteropolysaccharides (HePS). The HoPS group is composed of one type of monosaccharide, while the HePS group is composed of two or more different types of monosaccharides. Dextran is an α-glucan homopolysaccharide that contains only one type of monosaccharide, namely glucose with main α-1,6 glycosidic bonds with branches forming α-(1,2), α-(1,3) and α-(1,4) (Han et al. 2014; Iqbal et al. 2017). Several genera of LAB, such as *Leuconostoc, Streptococcus*, and *Lactobacillus* produce dextran by hydrolyzing sucrose (Saadat et al. 2019). According to Siddiqui et al. (2014), dextran exopolysaccharides are produced in large quantities by *L. mesenteroides*.

The dextran produced by *L. mesenteroides* was the first to be produced on an industrial scale and had broad benefits in the food and non-food fields (Han et al. 2014). Furthermore, its use in the pharmaceutical and medical fields includes cosmetic products, anticoagulants, immunomodulating drugs, blood plasma replacement components, increasing blood flow in capillaries, and anticoagulants (Nuwan et al. 2016; Stepanov et al. 2017). Dextran is used as an emulsifier, thickener, and gelling agent in the food industry.

Dextran biosynthesis by *L. mesenteroides* occurs because these bacteria produce extracellular dextransucrase enzymes that hydrolyze sucrose into glucose and fructose. The glucose is used to form intermediate compounds (glycosyl-enzyme) then polymerizes to form dextran, while the resulting fructose enters the bacterial cell through Phosphotransferases System (PTS) for used other metabolism (Diaz-Montes 2021). The degree to which dextran branches varies depending on the type of dextransucrase enzyme.

Dextran is synthesized by dextransucrase from microbial cells in the presence of sucrose as a substrate (Nuwan et al. 2016). The degree to which dextran branches varies depending on the type of dextransucrase enzyme. Furthermore, The amount of EPS produced by LAB depends on the type of microorganism, culture conditions and media composition (Zhou et al. 2017). Several studies showed that the concentration of added sucrose affects the synthesis of dextran from *L. mesenteroides*. Therefore, this research evaluated dextran production by *L. mesenteroides* strains isolated from lontar (*Borassus flabellifer* L.) sap.

MATERIALS AND METHODS

Procedures

Preparation of bacteria and inoculum

Leuconostoc mesenteroides N5 and *L. mesenteroides* N7 were isolated from lontar sap activated by growing on de Man Rogosa and Sharpe (MRS) agar (Merck, Germany) and incubated at 30°C for 48 h. The inoculum preparation was performed by transferring cultures of *L. mesenteroides* strains into 25 mL of MRS broth (Pronadisa, Spain) medium. Afterward, the cultures were incubated at 30°C, for 18 h at 100 rpm. The turbidity inoculum used has an optical density (OD) of 0.5 at 600 nm, equivalent to 109 CFU mL^{-1}.

Dextran fermentation and isolation

The production of dextran by both *L. mesenteroides* strains used of 100 ml MRS broth with the addition of sucrose (Pronadisa, Spain) at different concentrations of 5%, 10%, and 15% (w/v) then 10% (v/v) of inoculum was added. The fermentation was performed at 30°C, 100 rpm for 24 h. After fermentation, viability of LAB was analyzed and then the fermented broth was centrifuged at 6000 rpm for 20 min to remove the cells. Subsequently, the crude dextran was precipitated by adding cold ethanol (95%) as much as 2 times the filtrate volume and left at 4°C for 24 h to collect the precipitate (Nuwan et al. 2016) and the crude dextran determined by dry weight. Selected crude dextran from treatments was dialyzed using cut-off dialysis membrane 14 kD (Sigma-Aldrich, USA) in deionized water for 24 h with two changes, then dried by freeze drying for 18 h. The dialyzed dextran was analyzed for carbohydrate and reducing sugar content, protein content, functional group and surface morphology.

Viability of LAB

The viability of *L. mesenteroides* strains in fermented broth was conducted by total plate count. After which, 1 mL of the fermented broth was taken and added into 9 mL of sterile physiological saline. The serial dilutions were made up to 10^{-10}. Then *L. mesenteroides* strains were grown by pour plate using MRS agar medium and incubated aerobically at 30ºC for 48 h. The viable cell counts were expressed as colony forming units (CFU) per milliliter (Han et al. 2014).

Determination of total carbohydrate, reducing sugar, and protein

Total carbohydrate EPS was determined by the phenol sulfuric acid method using glucose as the standard (Dubois et al. 1956), reducing sugar was determined by 3,5-dinitrosalicylic acid (DNS) method (Miller 1959), while protein content was determined by the Lowry method using bovine serum albumin as the standard (Lowry et al. 1951).

Identification of dextran with Fourier Transform Infrared (FTIR)

The dextran was analyzed for its functional groups using FTIR. Dextran was mixed with dry KBr pellet and pressed in specimen. Compounds were analyzed at wave numbers 400-4000 cm^{-1} using 4 cm^{-1} (Shao et al. 2014). Dextran standard (Wako Pure Chemicals Tokyo, Japan) used has an average molecular weight of 32,000-45,000 Da.

Surface morphology of dextran with Scanning Electron Microscope (SEM)

The microstructure and surface morphology of the dextran was investigated by scanning electron microscopy. The dextran was fixed on aluminum stub and gold-sputtered before SEM examination maintaining an accelerated voltage of 10 kV (Farinazzo et al. 2019).

Data analysis

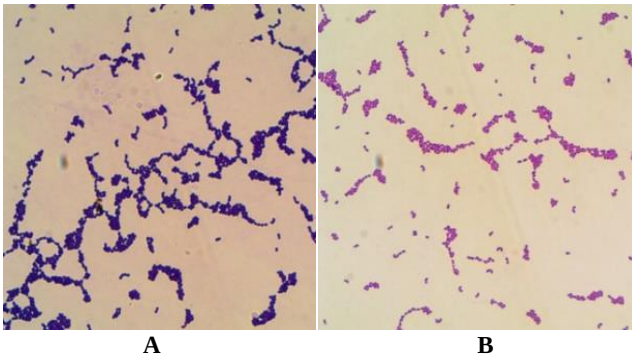
The data obtained with three repetitions were analyzed by two-way analysis of variance (ANOVA) with Tukey's post-hoc test with significance level ($P<0.05$) using the SPSS 23.0 program.

RESULTS AND DISCUSSION

Effect of sucrose concentration on dextran production by *Leuconostoc mesenteroides* strains

Exopolysaccharide-producing Lactic Acid Bacteria (LAB) isolated from lontar sap before use need to be reconfirmed by Gram staining and catalase test. The results showed that *L. mesenteroides* N5 and *L. mesenteroides* N7 were Gram positive, rod-shaped, and catalase negative. Gram stain of two *L. mesenteroides* strains are presented in Figure 1.

The ability of *L. mesenteroides* strains in dextran production was studied by fermentation in MRS broth with varying concentrations of 5, 10, and 15% (w/v). The amount of dextran production indicated the ability to use sucrose for dextran production by both of *L. mesenteroides* strains. The effect of sucrose concentration on the yield of dextran produced from both strains of *L. mesenteroides* is shown in Table 1. Furthermore, the type of strain and the concentration of sucrose significantly affected to dextran production ($P<0.05$). This result showed that different strains have different abilities to produce dextran. The amount of EPS produced is influenced by the microbial strain used (Seo et al. 2015). The interaction effect of strains and sucrose concentration showed no significance ($P>0.05$) on LAB viability.



A **B**

Figure 1. Gram staining of *Leuconostoc mesenteroides* N5 (A) and *L. mesenteroides* N7 (B)

The viability of LAB after fermentation showed that both *L. mesenteroides* strains grew well on medium with sucrose concentrations up to 15%. Furthermore, the number of living bacteria increased as the concentrations of sucrose increased. This showed that both strains are suitable for growing on medium containing high sucrose.

The dialyzed dextran produced by both strains of *L. Mesenteroides*, namely dextran N5 (*L. mesenteroides* N5) and dextran N7 (*L. mesenteroides* N7) showed in Figure 2. The characteristics of dextran N5 and dextran N7 are presented in Table 2. Both dextran N5 and N7 showed water-soluble properties. Results showed that total carbohydrates and reducing sugar in dextran N5 were 89.93% and 3.57%, whereas in dextran N7 was 87.84% and 3.76%. Dextran N5 and N7 had protein content of 2.33% and 2.22%, respectively.

Functional group of dextran

The results of FTIR spectra analysis (Figure 3) showed that dextran produced by both *L. mesenteroides* strains showed identical spectra. The dextran N5 and dextran N7 indicated the presence of carbohydrate structures, such as presence of hydroxyl vibrations, CH stretching, C=O stretching, bond vibrations (CH) (CH_2), covalent bond vibrations of COC and glycosidic bonds. The FTIR spectra is summarized in Table 3.

Surface structure of dextran

The surface structures of dextran N5 and dextran N7 were different (Figure 4). Dextran N7 exhibited porous network-like appearance that had smoother and shinier surface than dextran N5, while dextran N5 showed tighter morphology.

Table 1. EPS production by *Leuconostoc mesenteroides* strains

Strains	Sucrose conc. (%)	Yield of dextran (g/L)	Viability of LAB (CFU mL^{-1})
Leuconostoc mesenteroides N5	5	6.65 [a]	4.10^7
	10	8.80 [ab]	$3.3.10^8$
	15	14.53 [c]	$8.5.10^8$
Leuconostoc mesenteroides N7	5	6.22 [a]	$6.5.10^8$
	10	13.16 [bc]	$1.7.10^9$
	15	7.52 [a]	$8.3.10^9$

Table 2. Characteristics of dextran produced by the *Leuconostoc mesenteroides* strains

Characteristics	Dextran N5	Dextran N7
Color and texture	Brownish-yellow and crystalline	Brownish-yellow and crystalline
Solubility in water	Soluble	Soluble
Carbohydrate (%)	89.93	87.84
Reducing sugar (%)	3.57	3.76
Protein (%)	2.33	2.22

Table 3. FTIR spectra of dextran from *Leuconostoc mesenteroides* strains

Functional group	Wave number (cm^{-1})		
	Dextran N5	Dextran N7	Dextran standard
OH) stretching	3449	3433	3422
(CH) stretching	2940	2934	2930
(C=O)stretching	1636	1638	1642
(CH)(CH_2)	1345	1346	1345
COC vibration	1156	1156	1156
α -1,6 glycosidic bond	1009	1011	1013
α-glycosidic	914	914	914

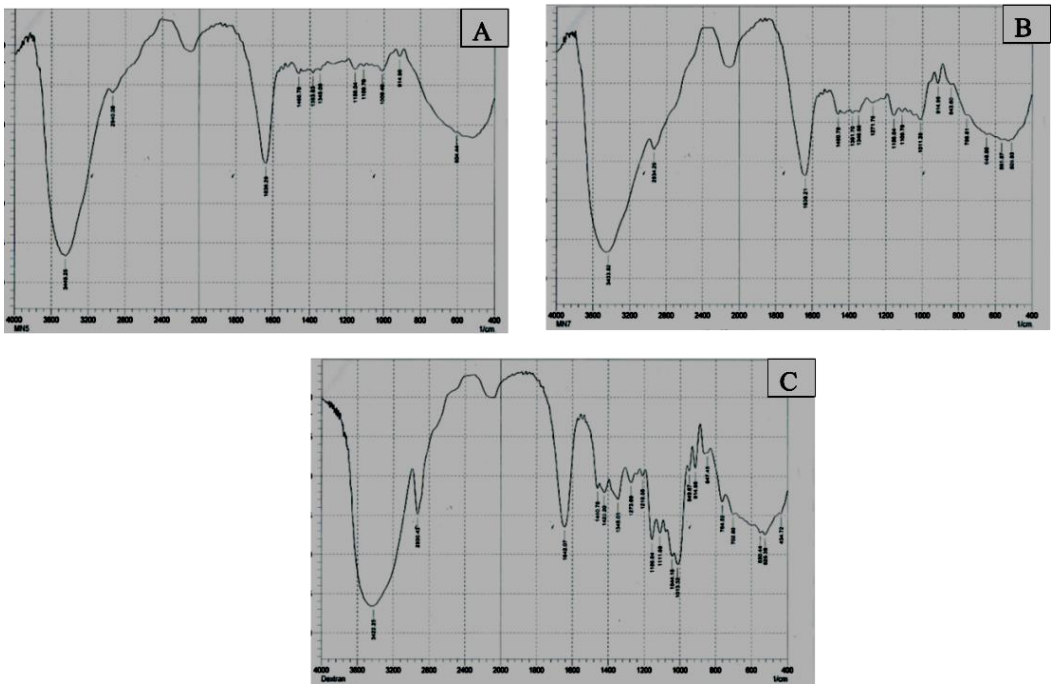
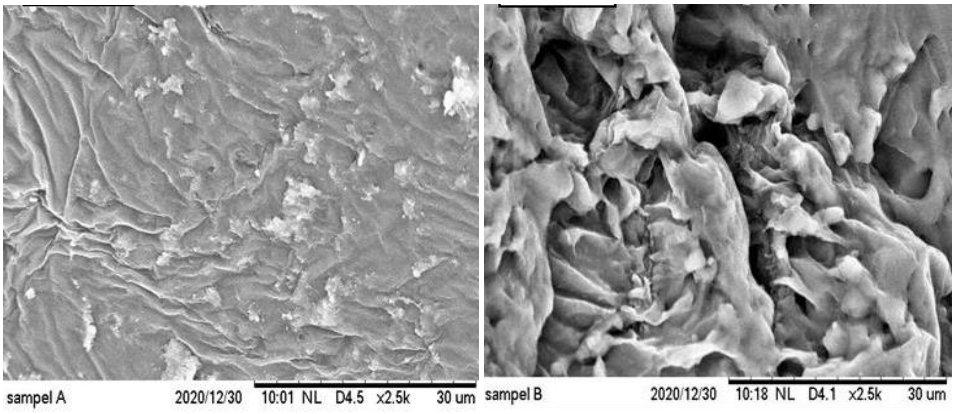


Figure 2. FTIR spectra of dextran N5 (A), dextran N7 (B) and dextran standard (C)



A **B**

Figure 3. Surface structure of dextran N5 (A) and dextran N7 (B) (2500x magnification)

Discussion

Leuconostoc mesenteroides N5 was able to produce higher dextran with an increase in the concentration of sucrose, and these results are in accordance with those reported by Nuwan et al. (2016). The highest dextran production by *L. mesenteroides* N5 was 14.53 g/L which was obtained on media with 15% sucrose. Meanwhile, *L. mesenteroides* N7 produced the highest dextran of 13.16 g/L with the addition of 10% sucrose. However, with 15% sucrose it failed to give the highest yield. This indicates an inhibitory effect of the substrate, which increases the production of dextran, and this is in accordance with the results of Sarwat et al. (2008) which showed a decrease in dextran production at high concentrations of sucrose.

The biosynthesis of dextran by *Leuconostoc* spp. showed that most of the fructose derived from sucrose is accumulated in the medium, while glucose was used as a substrate for the synthesis of dextran by the enzyme dextran sucrase (Han et al. 2014). Furthermore, the production of dextran by *L. mesenteroides* N7 at 5% and 15% sucrose concentrations were not significantly different, hence, the production of dextran using 15% sucrose was less efficient because a high sugar source was used. The highest *L. mesenteroides* N5 was $8.5.10^8$ CFU mL^{-1} in 15% sucrose medium, while *L. mesenteroides* N7 had a growth of $8.3.10^8$ CFU mL^{-1} which was higher than N5, but it was not accompanied by an increase in dextran production. This showed that a decrease in cell growth is not directly proportional to a decrease in EPS production.

The properties of dextran depend on the strain that produces it, therefore dextran has variations in molecular structure, molecular weight and branching that cause differences in its physical properties. Dextran N5 and N7 indicated water-soluble properties which showed that dextran had permeable polymer chain, hence, it binds large amounts of water through hydrogen bonds. The solubility and rheological properties of dextran are influenced by its molecular weight and branching (Guo et al. 2017). Dextran is a soluble type of EPS because of its ability to combine large amounts of water and form hydrogels (Campos et al. 2016).

Furthermore, the good solubility and water-holding ability of EPS make it an emulsifier or stabilizer for food products (Das et al. 2014). Total carbohydrates in dextran N5 and N7 were 89.93% and 87.84%, respectively. These results were in accordance with previous study of Siddiqui et al. (2014), who observed 83% carbohydrate content of dextran produced by *L. mesenteroides* KIBGE-IB22. The reducing sugar of dextran N5 and N7 were 3.57% and 3.76%, respectively. Sarwat (2008) reported that reducing sugar from dextran produced by *L. mesenteroides* CMG713 and *Leuconostoc mesenteroides* NRRL B512F were 1% and 25%, respectively. Meanwhile, the protein content of dextran N5 and N7 were 2.33% and 2.22%, respectively. This was due to the presence of media components that were contaminated in the polymer during the isolation process.

The results of FTIR analysis showed that the EPS produced by these two strains is a type of dextran that has identical spectrum to the standard dextran used. Previous research have shown that dextran produced by *L. mesenteroides* KIBGE-IB22 showed stretching vibrations of hydroxyl at 3430 cm^{-1}, stretching vibration of C-H at 2929 cm^{-1} and carboxyl at 1635 cm^{-1} (Siddiqui et al. 2014). Nuwan et al. (2016) reported that dextran produced by *L. mesenteroides* T1STR 053 showed OH stretching spectrum at 3438 cm^{-1}, CH vibration at 2924 cm^{-1}, CO stretching at 1637 cm^{-1} and α(1,6)-glycosidic at 1018 cm^{-1}. Based on the results of FTIR analysis, it showed that the dextran produced by both of two strains in this study have identical spectrum to the previous studies. Results also showed that the EPS produced by these two strains is a type of dextran which has identical spectrum to the dextran standard used.

The surface structure of the dextran in this study was similar to the dextran produced by *L. pseudomesenteroides* JF17 (Farinazzo et al. 2019) and *L. lactis* KC117496 (Saravanan and Shetty 2016) have matrix structure of porous polymer. The surface morphology of dextran confirmed that dextran N7 had good hydrophilicity, due to this hydrogen bonding it had greater water holding capacity than dextran N5. The difference in the surface structure of dextran also supported by the different constituent elements, namely that dextran N5 is less than dextran N7.

ACKNOWLEDGEMENTS

This work was supported by the Indonesian Ministry of Religious Affairs (Litapdimas Program).

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