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Effect of fermentation time on exopolysaccharide production by *Weissella confusa* isolated from peanut (*Arachis hypogaea* L.) milk

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ABSTRACT

Aims: The aim of this study was to identify an isolate B, an exopolysaccharide (EPS)-producing lactic acid bacteria and determine the fermentation time effect on EPS production.
Methodology and results: Isolate B, an EPS producer, was isolated from peanut milk containing commercial sugar, which was fermented spontaneously for 24 h. Isolate B was identified biochemically using API 50 CH and molecularly based on 16S rDNA. The effect of fermentation time on EPS production by isolate B with variations of the fermentation time were 12, 24, 36, 48 and 60 h. Isolate B was able to produce EPS qualitatively by producing mucoid colonies on solid media containing sucrose. The identification revealed that this isolate was *Weissella confusa* both biochemically using API 50 CH and molecularly based on 16S rDNA sequence homology-based method. The fermentation time significantly affected EPS production ($P<0.05$). Isolate B (*W. confusa*) produced the highest EPS (10.41 g/L) at 36 h with a cell viability of 6.5×10^8 CFU/mL. Furthermore, the FTIR results of EPS showed absorption bands characteristic of carbohydrates, including O-H, C-H, C=O, C-O-C and α-1,6 glycosidic groups. The EPS in this study was most likely a dextran type.
Conclusion, significance and impact of study: The yield of EPS production was influenced by fermentation time. Results suggest that *W. confusa* isolated from peanut milk had a good ability for EPS production. Therefore, it can be considered further to apply this strain for the production of EPS. However, further research is required.

Keywords: Exopolysaccharide, fermentation time, peanut milk, *Weissella confusa*

INTRODUCTION

Exopolysaccharide (EPS) is a biopolymer composed of sugar monomers secreted by microorganisms to protect cells from adverse environmental stresses, such as drying, osmotic pressure, pH, bacteriophages, toxic metal ions and antibiotics (Mıdık *et al*., 2020; Nguyen *et al*., 2020). In general, microorganisms do not use the polymer as an energy source but effectively protect microbial cells' integrity under stressful environmental conditions (Korcz and Varga, 2021). The increasing use of biopolymers in various fields has led to the development of research on the production of EPS by bacteria. Lactic acid bacteria (LAB) is an EPS-producing bacteria that is interesting to study because it is a microorganism in the Generally Recognized as Safe (GRAS) group. Therefore, the EPS produced is safe for consumption and has various structures (Nguyen *et al*., 2020).

EPS has various uses in the food sector, such as a thickener, stabilizer, or gelling agent, and has good water-binding ability (Stepanov *et al*., 2017). Meanwhile, EPS also has health effects, such as prebiotic, anti-tumor, antioxidant, immunomodulator, anticholesterol and antidiabetic (Wang *et al*., 2015). The EPS produced by LAB serves as a natural alternative to commercial food additives due to its physicochemical properties. The use of EPS in food products can provide the desired rheological changes in the food matrix, such as increased viscosity, reduced syneresis and better texture, while also having emulsifying, thickening, and stabilizing properties (Korcz and Varga, 2021).

There are two groups of EPS produced by LAB, namely homopolysaccharides (HoPs), which consist of repeating units of one monosaccharide type, such as D-glucose or D-fructose and heteropolysaccharides (HePs), which consist of two or more types of monosaccharides (Ryan *et al*., 2015; Caggianiello *et al*., 2016). The differences between HoPS and HePS are in their constituent monomers and structures, chain length, degree of branching and sugar linkages. HoPS usually consists of glucose or fructose units, each forming glucans or fructans. In general, glucans can be divided

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into four subgroups: dextrans, mutans, reuterans and alternans, while fructans consist of levan and inulin (Ryan *et al*., 2015; Korcz and Varga, 2021). The chemical structure of EPS affects its characteristic properties and physiological functions, so the structure of EPS needs to be studied, such as molecular weight, monosaccharides, composition, functional group, type of bond and content of non-carbohydrate substituents (Xu *et al*., 2018). The biosynthesis of EPS by bacteria is a complex process involving a number of enzymes and regulatory proteins. The biosynthesis of EPS by LAB occurs through two pathways, namely intracellular and extracellular. HoPs are generally produced by extracellular pathways, whereas HePs are produced through intracellular or extracellular pathways. The synthesis of HoPS is more straightforward when compared to the synthesis of HePS (Angelin and Kavitha, 2020).

Weissella confusa is a species of LAB that produces high amounts of EPS by adding a carbon source, such as sucrose, to the fermentation media. Environmental growth conditions, such as temperature, pH and incubation time, influence the EPS produced by the LAB. Also, it is influenced by media composition and growth factors (Mıdık *et al*., 2020). The production of EPS by *W. confusa* was extensively studied using de Man Rogosa and Sharpe (MRS) broth with the addition of sucrose. *Weissella confusa* requires complex nutritional requirements; hence, MRS media is widely used for culturing these bacteria. Several studies reported the production of EPS by these bacteria using MRS broth media with added sucrose, including *W. confusa* OF126 (Adesulu-Dahunsi *et al*., 2018) and *W. confusa* MD1 (Lakra *et al*., 2020).

The yield of EPS produced by LAB depends on the composition of the media and also on growth conditions such as fermentation time, temperature and pH. EPS is synthesized by LAB at different growth phases depending on the composition of the media and the type of microorganism that produces it; therefore, fermentation time is an essential factor in EPS production because of the specific ability of each strain of *W. confusa*. Besides that, the type of media used for production plays a vital role in determining production costs and it is necessary to find cheap media. This study evaluates the effect of fermentation time on EPS production by *W. confusa* isolated from peanut milk, which was not previously reported with the fermentation media other than MRS.

MATERIALS AND METHODS

Carbohydrate fermentation

Biochemical characterization was carried out by observing the fermentation profile of carbohydrates and their derivatives by isolate B using the API (Analytical Profile Index) 50 CH/CHL kit (Biomerieux, France) according to the instructions for use. Subsequently, the results of the fermentation test were identified using the Apiweb^{TM} software.

Molecular identification

Isolate B, which was grown for 48 h, was isolated by DNA using the Quick-DNA Fungal/Bacterial Miniprep Kit (Zymo Research, USA), according to the instructions for use. Furthermore, DNA suspension was confirmed using 0.8% (w/v) agarose gel electrophoresis. The DNA template was amplified by the polymerase chain reaction (PCR) method with 16S rDNA using primers 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-TAC GGY TAC CTT GTT ACG ACTT-3'). The obtained amplicons were confirmed by 1.5% (w/v) agarose gel electrophoresis. The sequence of nucleotide bases obtained was then analyzed using a sequence scanner, BioEdit and MEGA 6. The sequencing results were analyzed using the Basic Local Alignment Search Tool (BLAST) and compared with sequences in NCBI.

Bacterial and inoculum preparation

Weissella confusa was activated on de Man Rogosa dan Sharpe (MRS) agar (Merck, Germany) and incubated at 30 °C for 48 h. The inoculum was prepared by transferring several cultures of *W. confusa* into 25 mL of MRS broth (Merck, Germany) and incubated at room temperature (28 °C ± 2 °C) for 16 h at 100 rpm. The turbidity of the cell inoculum used for fermentation had an optical density (OD) of 0.5 at a wavelength of 600 nm, equivalent to 10^9 CFU/mL.

Production of EPS

The EPS production media used refers to Sarwat *et al*. (2008) with modifications. The media contains (g/100 mL) 10 g sucrose, 0.5 g peptone, 0.5 g yeast extract, 0.3 g K_2HPO_4, 0.005 g $CaCl_2 \cdot 2H_2O$, 0.001 g $MgSO_4 \cdot 7H_2O$ and 0.001 g NaCl. An amount of 5 mL of 5 mL *W. confusa* inoculum was taken and centrifuged at 5000 rpm for 15 min. Bacterial cells were collected, washed with sterile distilled water and centrifuged again. Subsequently, the cells were kept in 100 mL of EPS production media. The inoculated media were incubated at room temperature (28 °C ± 2 °C), 100 rpm, using various fermentation times (12, 24, 36, 48 and 60 h).

Extraction of EPS

EPS extraction refers to Adebayo-Tayo *et al*. (2018) with modified. The fermented media was centrifuged at 5000 rpm for 15 min to separate the cells. The collected filtrate was added with cold ethanol (Mallinckrodt, USA) two times the supernatant volume and allowed to settle at 4 °C for 24 h. The crude EPS precipitate was then separated by centrifugation at 5000 rpm for 20 min and dried at 60 °C until it reached a constant weight. The yield of EPS was expressed in grams of dry EPS per L of the amount of fermented media used for EPS extraction.

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Cell viability

Fermented media was taken in as much as 1 mL and diluted in 9 mL of sterile 0.85% NaCl solution from 10^{-1} to 10^{-8}. The dilution results were taken at 0.1 mL and planted into a Petri dish by pouring on a plate using MRS agar media. Subsequently, the plates were incubated at 37 °C for 48 h. The number of cell viability was expressed as colony-forming units (CFU) per milliliter (Han *et al.*, 2014; Ma'unatin *et al.*, 2022).

Identification of EPS with Fourier transform infrared (FTIR)

FTIR analysis was carried out on the selected EPS from the fermentation time that produced the highest yield. The analysis used the potassium bromide pellet method. The EPS was smoothed with KBr and analyzed in 4000-400 cm^{-1}. The data obtained through FTIR are in the form of functional groups or certain types of bonds at specific wave numbers (Adesulu-Dahunsi *et al.*, 2018).

Total carbohydrate and protein of EPS

The total carbohydrate of EPS was determined by the phenol sulfuric acid method using glucose as a standard (Dubois *et al.*, 1956) and protein content was determined by the Lowry method using bovine serum albumin as a standard (Lowry *et al.*, 1951).

Data analysis

The experiment was carried out with three repetitions. Quantitative analysis of the dependent variable was carried out using one-way ANOVA and further tested with Tukey's (P<0.05) using SPSS 23.0 for Windows (IBM).

RESULTS

Identification of isolate B

Isolate B was able to produce EPS on solid media, namely media fermentation for EPS production with the addition of agar. Figure 1 shows the ability of isolate B to form mucoid colonies on solid media containing 10% sucrose. This indicated that isolate B has good potential to produce EPS on the media. Increased EPS production can cause cell walls to become thicker. LAB synthesizes EPS to protect itself by retaining water around the cells and preventing dehydration. Furthermore, LAB synthesizes EPS to become a physical barrier on the cell surface, separating cells from environmental stresses so they can survive.

Initial identification of isolate B showed characteristics such as Gram-positive, rod cell shape, catalase, and negative endospores. Isolate B was biochemically identified at the species level using API 50 CH and molecularly based on 16S rDNA. In addition, API 50 CH test results are shown in Table 1. Meanwhile, PCR obtained a DNA amplicon of 1400 bp by molecular identification of Isolate B based on 16S rDNA (Figure 2). The results of molecular identification showed that isolate B was identified as *W. confusa* (Figure 3).

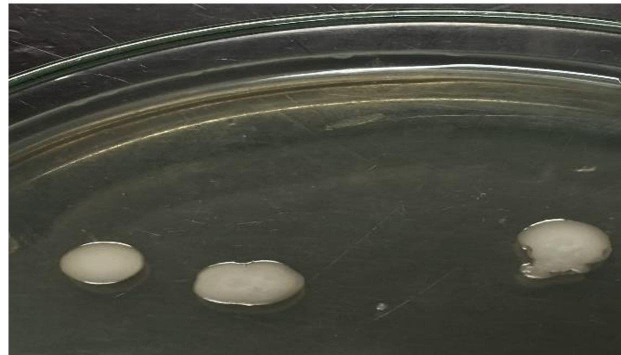

Figure 1: EPS production by isolate B on solid media containing sucrose.

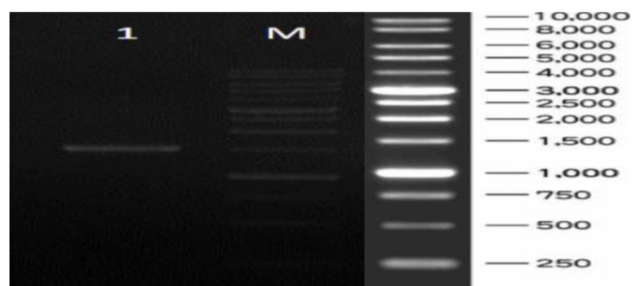

Figure 2: Results of PCR showing amplicons of 16S rDNA. 1, Isolate B and M, Marker.

Effect of fermentation time on EPS production by *W. confusa*

This study evaluated EPS production by *W. confusa* based on fermentation time using media with a carbon source of 10% sucrose. The presence of excess sugar in the media is able to induce the production of EPS due to osmotic stress. The results of EPS production by *W. confusa* at various fermentation times are shown in Table 2.

Fermentation time had a significant effect (P<0.05) on EPS production and cell viability of *W. confusa*. Furthermore, the fermentation time of 36 h showed that the highest EPS yield of 10.41 g/L was significantly different from other times (Table 1). The highest cell viability was obtained at 24 h of fermentation. The selected EPS obtained from fermentation for 36 h was used for further analysis. The EPS produced by *W. confusa* was yellowish-white and crystalline and composed of total carbohydrates and protein (91.77% and 0.64%, respectively) (Table 3). The production of EPS by *W. confusa* indicates a relationship between the production of EPS and cell viability.

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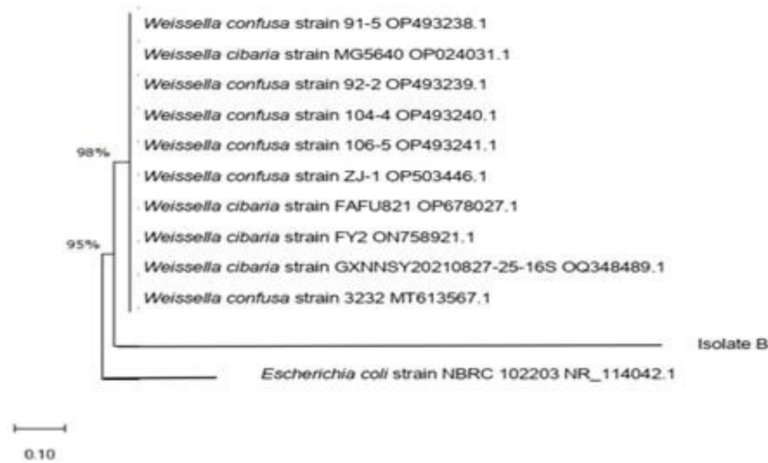

Figure 3: Phylogenetic tree of isolate B based on 16S rDNA.

Table 1: Fermentation profile of carbohydrates and their derivatives by isolate B.

Carbohydrates and their derivatives	Result	Carbohydrates and their derivatives	Result
Glycerol	-	Salicin	+
Erythritol	-	D-cellobiose	+
D-arabinose	-	D-maltose	+
L-arabinose	+	D-lactose	-
D-ribose	-	D-melibiose	-
D-xylose	+	D-sucrose	+
L-xylose	-	D-trehalose	-
D-adonitol	-	Inulin	-
Methyl-βD-Xylopranoside	-	D-melezitose	-
D-galactose	+	D-raffinose	-
D-glucose	+	Amidon (starch)	-
D-fructose	+	Glycogen	-
D-mannose	+	Xylitol	-
L-sorbose	-	Gentiobiose	-
L-rhamnose	-	D-turanose	-
Dulcitol	-	D-lyxose	-
Inositol	-	D-tagatose	-
D-mannitol	-	D- fucose	-
D-sorbitol	-	L-fucose	-
Methyl-αD- Mannopyranoside	-	D-arabitol	-
Methyl-αD-Glucopyranoside	-	L-arabitol	-
N-acetylglucosamine	+	Potassium gluconate	-
Amygdalin	+	Potassium 2-ketogluconate	-
Arbutin	+	Potassium 5-ketogluconate	-
Esculin	+		
Identification result: *Weissella confusa*			

Description: +, Positive reaction; -, Negative (no) reaction.

Table 2: Production of EPS by *W. confusa*.

Fermentation time (h)	EPS yield (g/L)	Cell viability (CFU/mL)
12	7.31 [ab]	1.4×10^{9} [a]
24	8.54 [c]	4.5×10^{10} [b]
36	10.41 [d]	6.5×10^{8} [a]
48	8.23 [bc]	5.4×10^{8} [a]
60	7.07 [a]	1.5×10^{8} [a]

Different letters show significant differences between treatments ($P<0.05$).

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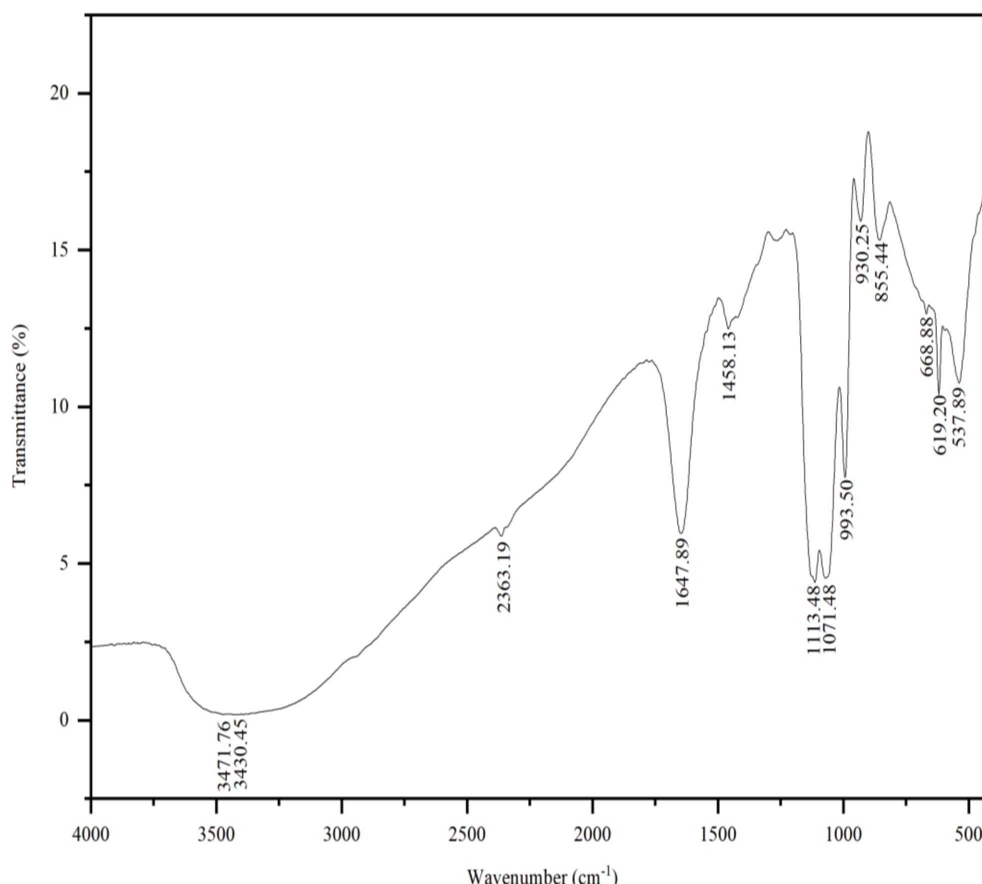

Figure 4: FTIR spectrum of EPS produced by *W. confusa*.

Table 3: Characteristics of EPS.

Characteristics	EPS
Color and texture	Yellowish-white and crystalline
Solubility in water	Soluble
Total carbohydrate (%)	91.77
Protein (%)	0.64

EPS functional group

The identified FTIR spectrum was used to determine EPS as a polysaccharide compound by qualitatively assessing the functional groups and types of bonds present in the EPS. The FTIR analysis of the selected EPS shows the absorption bands of carbohydrate compounds, a characteristic of exopolysaccharides (Figure 4).

DISCUSSION

The results of the biochemical fermentation test for carbohydrate compounds and their derivatives showed isolate B was able to ferment L-arabinose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, N-acetyl glucosamine, amygdalin, arbutin, esculin, salicin, D-cellobiose, D-maltose and D-sucrose. Based on the ability of isolate B to ferment these compounds, the identification results using API 50 CH showed that isolate B was *W. confusa*. The identification results of isolate B in this study were obtained using API 50 CH and molecularly based on 16S rDNA. Both methods' results were the same.

Table 1 shows that the EPS production increased on fermentation time from 12 to 36 h. *Weissella confusa* synthesized EPS with different amounts at various fermentation times. The highest production occurred at 36 h of fermentation time of 10.41 g/L, while EPS production at 48 and 60 h decreased. Cell viability did not reduce significantly at 36 to 60 h of fermentation, but the production of EPS decreased significantly. This study showed that the synthesis of EPS by *W. confusa* started in the initial period of fermentation, namely the logarithmic phase and continued to increase until the beginning of the death phase.

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The fermentation period of 12 to 24 h revealed a significant increase in the number of bacteria and an increase in the production of EPS. The highest cell viability at 24 h was 4.5×10^{10} CFU/mL. The fermentation time of 12 and 24 h were logarithmic phases, while that of 36 h showed the cell viability decreasing to 6.5×10^{8} CFU/mL. This study showed that the maximum production of EPS at 36 h did not follow the highest number of cell viability. This may be due to slow cell growth, and cell wall polymer synthesis was also slower, which makes more isoprenoid phosphate available for exopolymer synthesis (Wongsuphachat *et al*., 2010). Tayuan *et al*. (2011) also reported that the highest EPS production by *Weissella* sp. was obtained at 30 h of fermentation time, but the number of cell viability decreased. The maximum amount of EPS was obtained at 36 h while the highest cell viability was at 24 h; this result was similar to the research findings reported by Sánchez *et al*. (2006). After 36 h of fermentation, the EPS production and the number of bacteria decreased because the bacteria entered the death phase. Therefore, EPS was thought to be degraded by enzymes produced by bacteria and reused as a carbon source in the metabolic process. Several studies reported EPS degradation produced by LAB. The decrease in EPS yield was possible due to enzyme degradation. Glycohydrolase enzymes are able to hydrolyze EPS and release its monomers (Lin and Chien, 2007). The EPS produced by *W. confusa* in this study was similar to other strains from previous studies using MRS media with sucrose; namely, Lakra *et al*. (2020) produced an EPS of 10.07 g/L and higher than the 5.5807 g/L reported by Adebayo-Tayo *et al*. (2018), while Adesulu-Dahunsi *et al*. (2018) reported an EPS of 3 g/L.

EPS peaks at wave numbers 3471.76 and 3430.45 cm^{-1}, indicating abundant OH (hydroxyl) stretch vibrations. The wave number at 3438 cm^{-1} indicates the stretching of the hydroxyl compounds in the polysaccharides (Nuwan *et al*., 2016). The wave number 2363.19 cm^{-1} indicates a C-H stretching vibration. The peaks at wave numbers 1647.89 cm^{-1} and 1458.13 cm^{-1} showed C=O in carboxylates and aromatics. The peaks at wave numbers 1637.62 cm^{-1} and 1456.30 cm^{-1} were aldehydes and aromatics, respectively (Adebayo-Tayo *et al*., 2018). While wave numbers 1113.48 cm^{-1} and 1071.48 cm^{-1} were the presence of C-O-C and 1,6-glycosidic bonds. Iqbal *et al*. (2017) reported that the wave number of 1008.2-993.3 cm^{-1} indicated the presence of α-1,6 glycosidic bond. In addition, Lakra *et al*. (2020) reported that wave numbers 1024 cm^{-1} and 1020 cm^{-1} indicated the presence of 1,6 glycosidic bonds, which are typical of dextran-type EPS. Dextran produced by *Leuconostoc mesenteroides* KIBGE-IB22 showed the presence of hydroxyl at wave number 3430 cm^{-1}, C-H at 2929 cm^{-1}, and carboxyl at 1635 cm^{-1} (Siddiqui *et al*., 2014). Based on the FTIR results, it shows that the EPS produced by *W. confusa* in this study was likely to be a type of dextran. Previous studies have also reported that *W. confusa* produces dextran-type EPS (Heperkan *et al*., 2020).

Dextran is a homopolysaccharide type EPS composed of glucose monomers with α (1,6) bonds of linear chains and three different types of branched bonds (α-1,2; α-1,3 and α-1,4), the degree of dextran branching varies depending on the extracellular enzyme, which is a type of dextransucrase (Nuwan *et al*., 2016; Saadat *et al*., 2019). Several lactic acid bacteria produce this enzyme, namely *Leuconostoc*, *Lactobacillus*, *Streptococcus* and *Weissella*, using sucrose as the substrate.

CONCLUSION

The highest EPS produced by *W. confusa* isolated from peanut milk was 10.41 g/L with functional groups characteristic of carbohydrates. To obtain higher EPS yield, further study is required to optimize EPS production using other factors, such as media composition and growth conditions.

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REFERENCES

Adebayo-Tayo, B., Ishola, R. and Oyewunmi, T. (2018). Characterization, antioxidant and immunomodulatory potential on exopolysaccharide produced by wild type and mutant *Weissella confusa* strains. *Biotechnology Reports* **19, e00271.**

Adesulu-Dahunsi, A. T., Sanni, A. I., Jeyaram, K., Ojediran, J. O., Ogunsakin, A. O. and Banwo, K. (2018). Extracellular polysaccharide from *Weissella confusa* OF126: Production, optimization, and characterization. *International Journal of Biological Macromolecules* **111, 514-525.**

Angelin, J. and Kavitha, M. (2020). Exopolysaccharides from probiotic bacteria and their health potential. *International Journal of Biological Macromolecules* **162, 853-865.**

Caggianiello, G., Kleerebezem, M. and Spano, G. (2016). Exopolysaccharides produced by lactic acid bacteria: From health-promoting benefits to stress tolerance mechanisms. *Applied Microbiology and Biotechnology* **100(9), 3877-3886.**

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28(3), 350-356.**

Han, J., Hang, F., Guo, B., Liu, Z., You, C. and Wu, Z. (2014). Dextran synthesized by *Leuconostoc mesenteroides* BD1710 in tomato juice supplemented with sucrose. *Carbohydrate Polymers* **112, 556-562.**

Heperkan, Z. D., Bolluk, M. and Bülbül, S. (2020). Structural analysis and properties of dextran produced by *Weissella confusa* and the effect of different cereals on its rheological characteristics. *International Journal of Biological Macromolecules* **143, 305-313.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Iqbal, S., Marchetti, R., Aman, A., Silipo, A., Qader, S. A. U. and Molinaro, A. (2017). Enzymatic and acidic degradation of high molecular weight dextran into low molecular weight and its characterizations using novel Diffusion-ordered NMR spectroscopy. *International Journal of Biological Macromolecules* **103, 744-750.**

Korcz, E. and Varga, L. (2021). Exopolysaccharides from lactic acid bacteria: Techno-functional application in the food industry. *Trends in Food Science & Technology* **110, 375-384.**

Lakra, A. K., Domdi, L., Tilwani, Y. M. and Arul, V. (2020). Physicochemical and functional characterization of mannan exopolysaccharide from *Weissella confusa* MD1 with bioactivities. *International Journal of Biological Macromolecules* **143, 797-805.**

Lin, T. Y. and Chien, M. F. C. (2007). Exopolysaccharides production as affected by lactic acid bacteria and fermentation time. *Food Chemistry* **100(4), 1419-1423.**

Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry* **193(1), 265-275.**

Ma'unatin, A., Harijono, Zubaidah, E. and Rifa'i, M. (2022). Dextran production using *Leuconostoc mesenteroides* strains isolated from *Borassus flabellifer* sap. *Biodiversitas* **23(2), 1154-1158.**

Mıdık, F., Tokatlı, M., Elmacı, S. B. and Özçelik, F. (2020). Influence of different culture conditions on exopolysaccharide production by indigenous lactic acid bacteria isolated from pickles. *Archives of Microbiology* **202(4), 875-885.**

Nguyen, P. T., Nguyen, T. T., Bui, D. C., Hong, P. T., Hoang, Q. K. and Nguyen, H. T. (2020). Exopolysaccharide production by lactic acid bacteria: The manipulation of environmental stresses for industrial applications. *AIMS Microbiology* **6(4), 451-469.**

Nuwan, P., Piwpan, P., Jaturapiree, A. and Jaturapiree, P. (2016). Production of dextran by *Leuconostoc mesenteroides* TISTR 053 In fed batch fermentation. *KKU Research Journal* **22(1), 366-375.**

Ryan, P. M., Ross, R. P., Fitzgerald, G. F., Caplice, N. M. and Stanton, C. (2015). Sugar-coated: Exopolysaccharide producing lactic acid bacteria for food and human health applications. *Food & Function* **6(3), 679-693.**

Sánchez, J., Martínez, B., Guillén, R., Jiménez-Díaz, R. and Rodríguez, A. (2006). Culture conditions determine the balance between two different exopolysaccharides produced by *Lactobacillus pentosus* LPS26. *Applied and Environmental Microbiology* **72(12), 7495-7502.**

Saadat, Y. R., Khosroushahi, A. Y. and Gargari, B. P. (2019). A comprehensive review of anticancer, immunomodulatory and health beneficial effects of the lactic acid bacteria exopolysaccharides. *Carbohydrate Polymers* **217, 79-89.**

Sarwat, F., Qader, S. A. U., Aman, A. and Ahmed, N. (2008). Production and characterization of a unique dextran from an indigenous *Leuconostoc mesenteroides* CMG713. *International Journal of Biological Sciences* **4(6), 379-386.**

Siddiqui, N. N., Aman, A., Silipo, A., Qader, S. A. U. and Molinaro, A. (2014). Structural analysis and characterization of dextran produced by wild and mutant strains of *Leuconostoc mesenteroides*. *Carbohydrate polymers* **99, 331-338.**

Stepanov, N. A., Senko, O. V. and Efremenko, E. N. (2017). Biocatalytic production of extracellular exopolysaccharide dextran synthesized by cells of *Leuconostoc mesenteroides*. *Catalysis in Industry* **9(4), 339-343.**

Tayuan, C., Tannock, G. W. and Rodtong, S. (2011). Growth and exopolysaccharide production by *Weissella* sp. from low-cost substitutes for sucrose. *African Journal of Microbiology Research* **5(22), 3693-3701.**

Wang, J., Zhao, X., Tian, Z., He, C., Yang, Y. and Yang, Z. (2015). Isolation and characterization of exopolysaccharide-producing *Lactobacillus plantarum* STK109 from Tibet Kefir. *Polish Journal of Food and Nutrition Sciences* **65(4), 269-279.**

Wongsuphachat, W., Kittikun, A. H. and Maneerat, S. (2010). Optimization of exopolysaccharides production by *Weissella confusa* NH 02 isolated from Thai fermented sausages. *Songklanakarin Journal of Science and Technology* **32(1), 27-35.**

Xu, Z., Guo, Q., Zhang, H., Wu, Y., Hang, X. and Ai, L. (2018). Exopolysaccharide produced by *Streptococcus thermophiles* S-3: Molecular, partial structural and rheological properties. *Carbohydrate Polymers* **194, 132-138.**