HAYATI
Journal of Biosciences

Vol. 31 No. 3, May 2024 539-547
DOI:10.4308/hjb.31.3.539-547
ISSN: 1978-3019
EISSN: 2086-4094

The Potential of *Weissella confusa* K2 Isolated from Longan (*Dimocarpus longan*) Fruit as High Exopolysaccharide-Producing Strain

Anik Ma'unatin*, Afifatul Fitri Khoirumiyah

Department of Chemistry, Faculty of Science and Technology, Universitas Islam Negeri Maulana Malik Ibrahim, Malang 65144, Indonesia

ARTICLE INFO

Article history:
Received June 9, 2023
Received in revised form December 26, 2023
Accepted December 29, 2023

KEYWORDS:
carbon source,
exopolysaccharide,
longan fruit,
pH,
Weissella confusa

ABSTRACT

Isolation of Lactic Acid Bacteria (LAB) from various sources has previously been carried out to obtain strains that produce high of exopolysaccharide (EPS). Isolate K2, which was LAB isolated from longan (*Dimocarpus longan*) fruit, can produce EPS. This study aimed to identify isolate K2, and evaluated the effect of supplementation carbon sources (glucose, fructose, sucrose, and lactose) and pH of media (4, 5, 6, 7, and 8) on EPS production. Isolate K2 was identified as *Weissella confusa* molecularly based on 16S rDNA, the type of carbon source, and the pH of media had significant effects (P<0.05) on EPS production. *W. confusa* K2 produced EPS that was highest on media with the supplementation of sucrose as a carbon source and produced the lowest EPS on lactose. The amount of EPS produced by *W. confusa* K2 under alkaline was higher than under acidic, the highest EPS of 47.703 g/L at pH 8 and the lowest EPS at pH 4 of 1.858 g/L. The physical properties showed EPS has good solubility in water with the solubility and Water Holding Capacity (WHC) at 40.533% and 354% respectively. Based on Surface morphology of EPS with Scanning Electron Microscope (SEM) analysis, the surface structure of EPS was a porous polymer matrix. Fourier Transform Infrared Spectrophotometer (FTIR) results of EPS showed the presence of absorption bands as characteristic of carbohydrates, such as the presence of O-H, C-H, C=O, C=C, C-O-C groups, and glycosidic bonds.

1. Introduction

The use of biopolymers in various fields has increased in recent years, led to the development of research on the production of exopolysaccharides by bacteria. Exopolysaccharide (EPS) is a high molecular mass polymer with long chains produced through the metabolic pathways of microorganisms (Wang *et al.* 2015) among other lactic acid bacteria (LAB). LAB is EPS-producing bacteria that has attracted attention because LAB is known as a safe microorganism (GRAS-Generally Recognized As Safe) therefore, the EPS is as safe as its metabolites (Feng *et al.* 2018). Several LAB genera often used for EPS production are *Lactobacillus*, *Leuconostoc*, *Streptococcus*, *Weissella* and *Lactococcus* (Kavitake *et al.* 2020).

The diversity of EPS structures capable of being produced by LAB has special attention in the food and pharmaceutical sector. LAB can produce various kinds of EPS with different chemical structures and physical characteristics, and this can affect its application in the industry (Prete *et al.* 2021). Several studies have shown that the benefits of EPS have a promising future. EPS has very good health effects including as an antioxidant, anticancer, antiviral, anti-inflammatory, and also has the effect of lowering cholesterol. EPS can also be used as a thickener, stabilizer and emulsifier in the food industry (Nguyen *et al.* 2020).

The EPS produced by LAB has 2 groups, namely homofermentative (HoPS) and heterofermentative (HePS). EPS with the HoPS structure consists of one type of monosaccharide. The HoPS produced by LAB consists of repeating units of monosaccharides. HoPS can be classified into 2 groups, namely α-D-glucan (dextran, mutant, reuteran, and alternan) and fructans (levan and inulin) (Saadat *et al.* 2019). HePS is EPS, which consists of several types of

* Corresponding Author
E-mail Address: anik.maunatin@uin-malang.ac.id

monomers. The type of HePS produced by LAB is kefiran. BAL synthesizes EPS at different growth phases depending on the media composition and type of microorganism it produces. EPS biosynthesis by bacteria is a complex process involving many enzymes and regulatory proteins (Patel *et al.* 2012). EPS biosynthesis in LAB occurs in two ways, namely intracellular and extracellular. Homopolysaccharide EPS is generally produced through extracellular biosynthetic pathways, while heteropolysaccharide EPS is synthesized through intracellular or extracellular pathways (Angelin and Kavitha 2020).

EPS has an important role in controlling the physicochemical characteristics of the cell surface (Polak-Berecka *et al.* 2014). EPS is synthesized by bacteria with various functions, including protecting bacteria to survive under stressful conditions (Nguyen *et al.* 2020). EPS production is generally considered a response strategy used by microorganisms to resist stress from the environment. The yield of EPS production depends on many factors, such as the cultivation condition, carbon source, C/N ratio, pH of media, and cultivation time (Netrusov *et al.* 2023).

The type of carbon sources and the pH were among the most important environmental parameters critical for the biosynthesis of EPS produced by bacteria (Ju *et al.* 2022). The pH of the fermentation media was one of the most critical environmental parameters for bacterial exopolysaccharide biosynthesis, changes in the pH of the media affect the production of exopolysaccharides produced by bacteria and their molecular weight (Ju *et al.* 2022). The optimum pH for EPS production varies depending on the LAB strain used and the culture conditions. Some studies showed that there was a correlation between the pH of the media and EPS production, low pH increased EPS production (Slížová *et al.* 2015; Sanhueza *et al.* 2015; Nguyen *et al.* 2021), however, there were also reported that low pH decreased EPS production (Tayuan *et al.* 2011). Each LAB species requires a type carbon source and specific of pH media stress conditions to obtain the high yield of EPS, so these two factors are very important to study in efforts to increase the production EPS.

Isolate K2 was LAB isolated from longan (*Dimocarpus longan*) fruit, which fermented spontaneously in MRS media and was able to produce EPS. EPS production by LAB from longan fruit has not been reported therefore, it is necessary to study the ability of K2 isolates to produce EPS. This study aimed to identify isolate K2 and evaluated the effect of adding carbon sources and pH of media on EPS production, therefore the results of this study can be used to determine the suitable of carbon source and pH for the production of high-yield EPS. EPS was also characterized by physico-chemical.

2. Materials and Methods

2.1. Morphological and Biochemical Characteristics

Single colony of isolate K2 that has been grown on de Man Rogosa and Sharpe (MRS) agar (Merck, Germany) was observed based on morphological characteristics, then was followed by a biochemical characterization test based on Bergey's Manual of Determinative Bacteriology (Holt *et al.* 1994) including tests such as Gram-staining, catalase, endospore, motility, production of (catalase, oxidase, coagulase and urease), other biochemical tests and carbohydrate fermentation.

2.2. Molecular Identification of EPS-Producing Lactic Acid Bacteria (LAB)

Isolate K2 was grown for 48 hours on de Man Rogosa and Sharpe (MRS) agar. DNA was isolated using a commercial DNA kit according to the instructions for use. Suspension DNA was confirmed using 0.8% (w/v) agarose gel electrophoresis. The DNA template was amplified by polymerase chain reaction (PCR) method with 16S rDNA using 27F primer (5`- AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5`TAC GGY TAC CTT GTT ACG ACTT-3'). The obtained amplicon was confirmed by 1.5% (w/v) agarose gel electrophoresis. Nucleotide base sequences were analyzed using a sequence scanner, BioEdit, and MEGA 6 software. Results sequencing analyzed using the Basic Local Alignment Search Tool (BLAST) was compared with sequences at NCBI.

2.3. Inoculum Preparation

Isolate K2 was grown on MRS agar and incubated at 30°C for 48 hours. Inoculum was prepared by transferring several oses of culture into MRS broth (Merck, Germany) and incubated at 30°C, 100 rpm for 18 hours. The inoculum used for fermentation had cell turbidity, namely optical density (OD) 0.5 at a wavelength of 600 nm, equivalent to 10^{10} CFU/ml.

2.4. Effect of Carbon Sources on EPS Production

1 ml of inoculum was added to 9 ml of supplemented MRS media with different carbon sources 10% (w/v). The carbon sources used were glucose (Merck, Germany), fructose (Himedia, India), sucrose (Phytotech, USA) and lactose (Merck, Germany). MRS media without additional carbon sources was used as control. Incubation was carried out at room temperature (28°C±2°C), 100 rpm for 24 hours. Then isolation and quantification of EPS were carried out.

2.5. Effect of pH on Exopolysaccharide Production

The effect of the initial pH of media on EPS production was evaluated with pH variations (4, 5, 6, 7 and 8). 100 ml of MRS media supplemented with 10% (w/v) of sucrose was adjusted to the pH using HCl 2 N and NaOH 2 N before sterilization. The media was added 5 mL of inoculum and then incubated at room temperature (28°C±2°C), 100 rpm for 24 hours. Then isolation and quantification of EPS were carried out.

2.6. EPS Isolation and Quantification

EPS extraction refers to Wang *et al.* (2015b) and Adebayo-Tayo *et al.* (2018) with modification. The fermented culture was heated at 100°C for 20 minutes, then 10% (v/v) TCA (Merck, Germany) was added with a ratio of 1:1 and shaken for 30 minutes, then centrifuged at 5,000×g for 15 minutes at 4°C. The supernatant was removed and precipitated with cold absolute ethanol (Merck, Germany) as much as 2 times the volume of fermented media and left for 24 hours at 4°C for EPS precipitation. The precipitate of EPS was taken by centrifugation 5,000×g for 20 minutes at 4°C. The pellets obtained were dried at 60°C until the weight was constant, then the EPS was weighed, and the yield was determined.

2.7. Estimation of Carbohydrate and Protein Content of EPS

The total carbohydrate content of EPS was determined by the phenol sulfuric acid method (Dubois *et al.* 1956). The protein content in EPS was estimated by the Folin-Lowry method (Lowry *et al.* 1951).

2.8. Solubility and Water Holding Capacity (WHC)

Solubility was determined by making 25 mg/ml of EPS solution in deionized water, stirring continuously at 30°C for 24 hours, and centrifugation at 5,000×g for 15 minutes. The supernatant was taken 0.5 ml and added 3 times the volume of absolute ethanol. EPS was recovered by centrifugation at 5,000×g for 15 minutes. The precipitate was dried at 60°C. Solubility was calculated using the formula.

$$\text{Solubility (\%)} = \frac{\text{Total carbohydrate concentration in supernatant}}{\text{Dry weight of sample}} \times 100$$

Water holding capacity (WHC) was determined by preparing 40 mg/ml EPS in deionized water and mixing, then centrifuged at 5,000 rpm for 20 minutes. The precipitate obtained was placed on filter paper which had been weighed before to remove water that was not bound by EPS. Then EPS was weighed and WHC was calculated based on the formula described previously (Sharma *et al.* 2020).

$$\text{WHC (\%)} = \frac{\text{Total sample weight after water absorption}}{\text{Dry weight of sample}} \times 100$$

2.9. Surface morphology of EPS with Scanning Electron Microscope (SEM)

The surface morphology of EPS was observed by scanning electron microscopy. The EPS was fixed on an aluminum stub and gold-sputtered before SEM examination, maintaining an accelerated voltage of 10 kV (Farinazzo *et al.* 2019; Ma'unatin *et al.* 2022).

2.10. Fourier Transform Infrared Spectrophotometer (FTIR) of EPS Analysis

FTIR analysis was performed using the potassium bromide pellet method. EPS was smoothed with KBr and analyzed in the area of 4000-400 cm^{-1}. The data obtained through FTIR was in the form of certain functional groups or bond types at certain wave numbers (Adesulu-Dahunsi *et al.* 2018).

2.11. Statistical Analysis

The experiment was carried out with three repetitions. The results of the EPS yield were analyzed

using analysis of variance (ANOVA) and further testing with Tukey's post-hoc test using SPSS 23.0 for Windows (IBM).

3. Results

3.1. Identification of Isolate K2

This strain was observed as Gram-positive rod-shaped, endospore and catalase-negative, and non-motile bacteria. Isolate K2 was a group of lactic acid bacteria. Based on the results of the fermentation test of carbohydrate compounds showed isolate K fermented glucose, mannitol, xylose, rhamnose, sucrose, lactose, arabinosa, adonitol, raffinose, and salicin. Morphological and biochemical characteristics of isolate K2 are presented in Table 1. Isolate K2 produced EPS qualitatively on MRS agar media supplemented with sucrose (Figure 1C).

Isolate K2 was identified at the species level based on 16S rDNA. This study showed molecular identification of K2 isolate based on 16S rDNA was obtained the DNA amplicon had a size of 1,500 bp. Sequencing analysis showed that the strain was phylogenetically closely related to the genus *Weissella*. Blast analysis of 16S rDNA sequences showed that isolate K had a maximum similarity of 99% with *Weissella confusa* (Figure 2).

3.2. Effect of Carbon Sources on EPS Production

Based on the results of ANOVA showed that the type of carbon source had a significant effect ($P<0.05$) on EPS

Table 1. Morphological and biochemical characteristics of isolate K2

Characteristics	Results
Culture characteristics (color, shape, margins, surface, elevation, colony diameter)	circular, flat/entire, smooth, convex, 2.19 mm
Gram's reaction	G+
Cell morphology	rod
Endospore	-
Motility	-
Production from :	-
Catalase	-
Oxidase	-
Coagulase	-
Urease	-
Biochemical Characteristics:	
Nitrate	-
H_2S	-
Indole	-
Voges-Proskauer	-
Citric	-
gelatin	-
Carbohydrate fermentation:	
Glucose	+
mannitol	+
xylose	+
Rhamnose	+
sucrose	+
Lactose	+
Arabinose	+
Adonitol	+
Raffinose	+
Salicin	+
Starch hydrolysis	+
Casein hydrolysis	+

Description: + = positive reaction; - = negative reaction

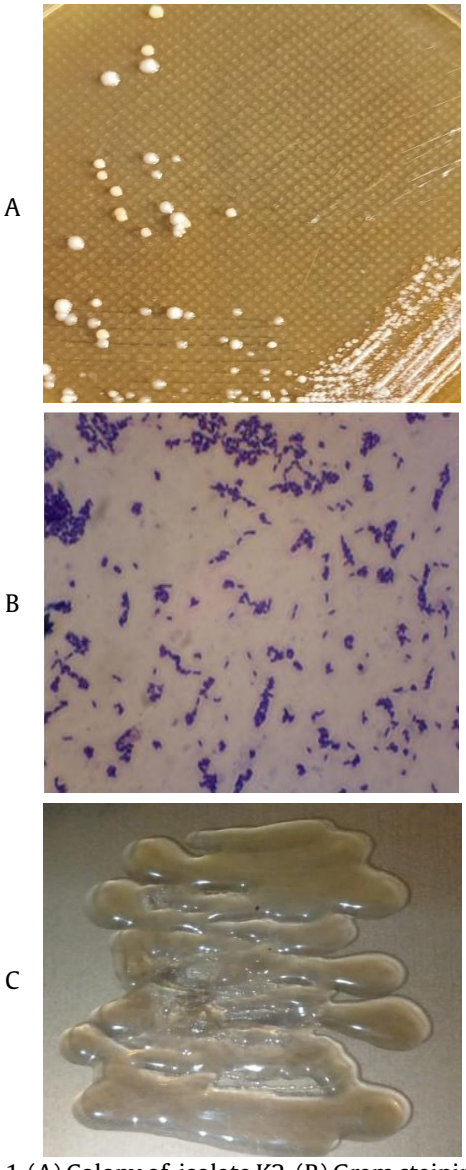


Figure 1. (A) Colony of isolate K2, (B) Gram staining (1,000x magnification), (C) EPS production by isolate K2 on agar media

yield. Table 2 showed that supplementation of different carbon sources on the MRS media caused differences in EPS yield. The highest EPS yield was 13.103 g/L, which was achieved by adding sucrose as a carbon source. Other carbon sources, namely glucose, fructose, and lactose, had no significant effect on the EPS yield, however the addition of sucrose produced significant EPS with other carbon sources. Supplementation of carbon sources sequentially capable of producing EPS from low to high was glucose, lactose, fructose and sucrose. Sucrose was the best carbon source in EPS production by *W. confusa* K2 from longan, so sucrose was used as a source of sugar for further fermentation.

3.3. Effect of pH on Exopolysaccharide Production

EPS production by *W. confusa* K2 was increased by evaluating the effect of initial pH using MRS media with supplementation of sucrose 10% (w/v). The EPS yield increased as the initial pH of the fermentation media increased. Therefore, in acidic media conditions, EPS decreased drastically (Table 3). Observations of changes in the viscosity of the media after fermentation visually showed that the higher the initial pH, the higher the viscosity of the media, this is due to the higher concentration of EPS formed. The initial pH of media had a significant effect ($P<0.05$) on the EPS yield produced by *W. confusa* K2, where pH 8 showed the highest EPS yield of 47.703 g/L and significantly different from the other pH, while the lowest EPS was obtained at a low pH of 4, which produces EPS of 1.858 g/L. Stress condition media at pH 8 stimulated *W. confusa* K2 to synthesize high EPS. Selected EPS obtained from pH 8 production media was used for further analysis.

Table 2. Production of EPS with the addition of carbon sources

Carbon sources	EPS yield (g/L)
MRS (control)	0.723[a]
Glucose	1.173[a]
Fructose	1.850[a]
Sucrose	13.103[b]
Lactose	1.163[a]

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

Table 3. Production of EPS with pH variations

pH	EPS yield (g/L)
4	1.858[a]
5	3.507[a]
6	21.217[b]
7	41.096[c]
8	47.703[d]

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

Figure 2. Phylogenetic tree of isolate K2 based on 16S rDNA

3.4. Physico-chemical Characteristics of EPS

The selected EPS comprised total carbohydrate and protein 81.477% and 1.006% respectively. While the solubility in water and WHC of EPS were 40.533% and 354% respectively. The physicochemical characteristics of the selected EPS produced by *W. confusa* K2 are presented in Table 4. The surface structure of EPS is shown in the form of network as a matrix of porous polymer (Figure 3B).

3.5. Functional Group Analysis of Exopolysaccharides with FTIR Spectrophotometer

FTIR spectra analysis determined the main functional groups and chemical bonds found in EPS. EPS produced by LAB is a complex polysaccharide containing various functional groups. The FT-IR spectrum of this EPS from *W. confusa* K2 as shown in Figure 4 had peaks from 3427 to 418 cm^{-1}. The FTIR spectra of selected EPS in this study observed that EPS had functional groups and bond types characteristic of polysaccharide compounds, including O-H, C-H, C=O, C-O-C, and α-1,6 glycosidic groups. The EPS from *W. confusa* K2 was likely a dextran type.

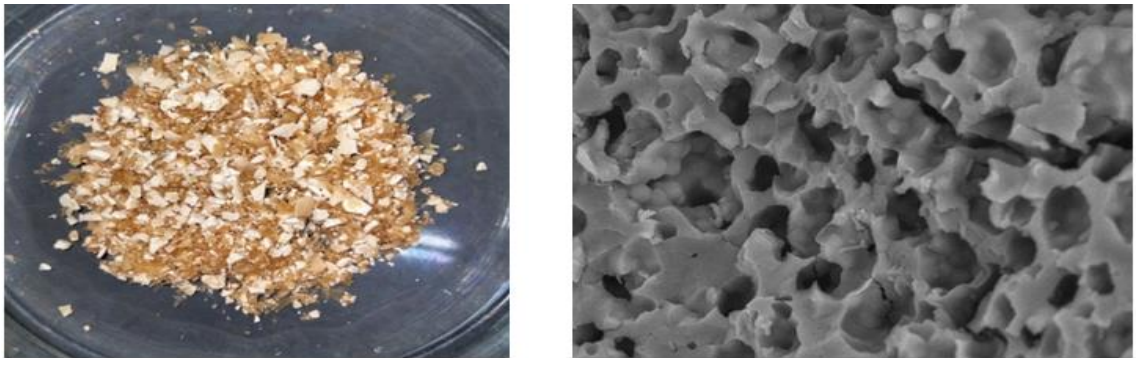
Table 4. Physico-chemical characteristics of EPS

Characteristics	EPS
Color and texture	Light brown and crystalline
Total carbohydrate (%)	81.477
Protein (%)	1.006
Solubility in water	Soluble
Solubility (%)	40.533
WHC (%)	354

4. Discussion

Identification of isolate K2 showed general characteristics as a group of lactic acid bacteria namely Gram-positive, catalase, and endospore-negative. Isolate K2 isolated from this longan fruit was identified molecularly as *Weissella confusa*. Based on EPS production, *W. confusa* K2 could use different carbon sources with different stimulating effects on EPS production. The highest EPS yield was obtained in addition to sucrose. The addition of glucose causes a slight increase in production EPS when compared to the control, namely MRS which contains 20 g/L glucose, the high glucose content in the media does not affect to EPS yield. Glucose and fructose are monosaccharides that play a role in the glycolytic and pentose phosphate pathways. Besides that, another type of disaccharide sugar, lactose, produced smaller EPS when compared to sucrose. This study showed differences in carbon sources have different effects on the catabolic expression of metabolite. This result was similar to reported by Adesulu-Dahunsi *et al.* (2018) that *W. confusa* produced the highest EPS in addition to sucrose carbon source than glucose, lactose, and galactose. This study also supported that *W. confusa* could produce high EPS when using sucrose as a carbon source.

The initial pH of media under acidic and alkaline conditions caused significant differences in EPS production by *W. confusa* K2. Acidic media decreased EPS yield, while in alkaline media, EPS increased Drastically. This study showed EPS production depends



A B

Figure 3. (A) EPS product and (B) surface morphology of EPS from *W. confusa* K2 (2,500x magnification)

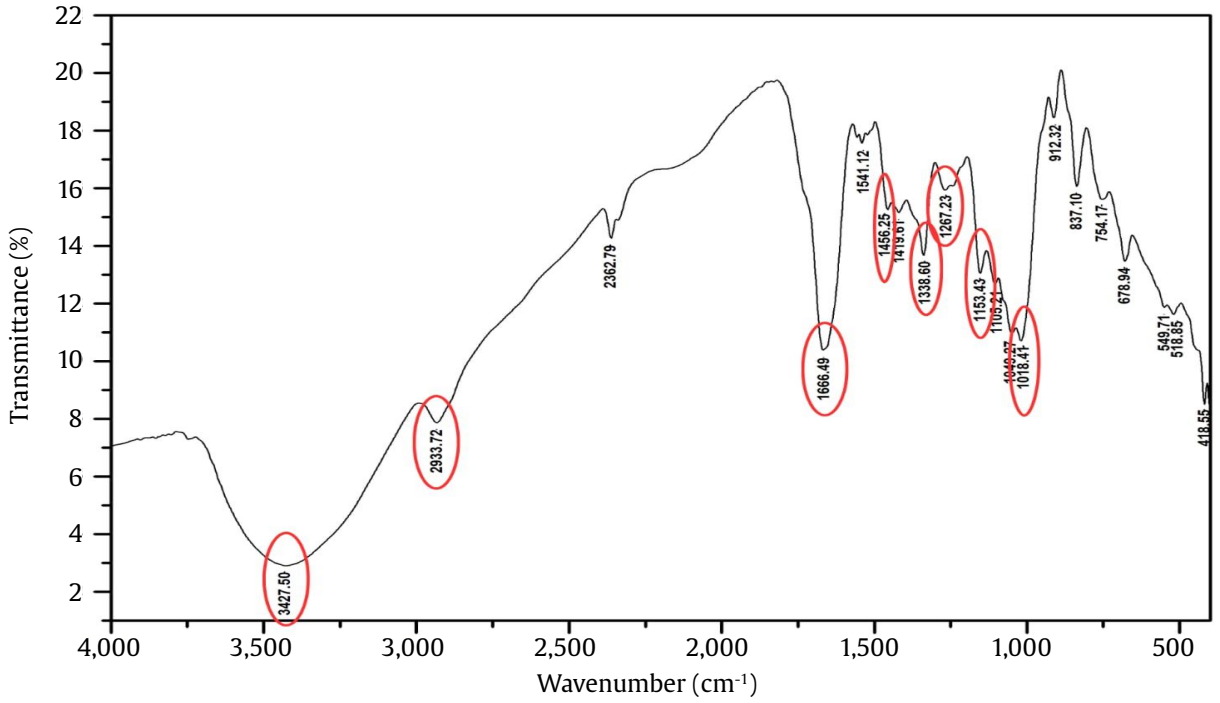


Figure 4. FTIR spectra of EPS produced by *W. confusa* K2

on the initial pH of media. The lowest EPS yield at pH 4, this was presumably because the high level of acidity of the media caused acid stress in the cells, whereas at pH 8 produced the highest of EPS. The results of previous studies similar to this study have also been reported. The highest EPS production by *Weissella* sp. of 8.65 g/L was achieved in culture with an initial pH of 7.0 (Tayuan *et al.* 2011). In addition, previous studies also reported that *W. confusa* strain produced the highest EPS in acidic media, including Dubey and Jeevaratnam (2015) reported that *W. confusa* AJ53 produced the highest EPS of 1.29 g/L at pH 6.5, *W. confusa* MD1 produced the highest EPS, namely 10.07 g/L at pH 6.5 (Lakra *et al.* 2020). *W. confusa* K2 produced a high EPS of 47.703 g/L therefore this study reported that *W. confusa* K2 as a strain isolated from longan fruit, had good potential for EPS production.

The FTIR results on EPS showed that there was extensive stretching in the region of 3427 cm^{-1} which indicates the presence of abundant O-H strain vibrations, this was the characteristic absorption band carbohydrate ring and was responsible for the solubility of EPS in water (Wang *et al.* 2010). Weak spectra at 2933 cm^{-1} associated with C–H stretching vibrations. Spectra in the region of 1666 cm^{-1} was the stretching characteristic of C=O and carboxyl, which showed aldehyde, absorption band 1456 cm^{-1} indicated aromatic (Adebayo-Tayo *et al.* 2018), presence covalent bond of C-O-C at 1153 cm^{-1} (Ma'unatin *et al.* 2022), absorption band 754 cm-1 indicated the presence of glycosidic bonds of polysaccharide (Saravanan and Shetty 2016). FTIR spectrum analysis revealed that EPS was a complex polysaccharide structure containing different functional groups.

EPS produced by *W. confusa* K2 was able to dissolve in water and the good of WHC namely 40.533% and 354%, respectively. These properties were related to the permeable structure of the polymer chain, which retain large amounts of water through hydrogen bonds. According to Tingirikari *et al.* (2014) *W. cibaria* JAG8 produced dextran, which had water soluble index of 24.5% and WHC of 352%. The good solubility and water-holding capacity of EPS in this study made it possible for applications in food products, such as water-binding agents and stabilizers.

Observation using SEM showed the three-dimensional morphology and surface structure of EPS so that it helped in understanding its physical properties. Based on the surface morphology of EPS confirmed that the surface structure showed porous network caused it had good hydrophilicity which correlated with the high solubility and WHC of this EPS. EPS which was soluble in water with good WHC, this was related to the matrix structure porous which

could hold large amounts of water through hydrogen bonds (Zhou *et al.* 2017). The surface of the EPS in this study was similar to the dextran produced by *Leuc. pseudomesenteroides* JF17 which was porous (Farinazzo *et al.* 2019), Dextran N7 produced by *Leuconostoc mesenteroides* N7 exhibited a porous surface structure (Ma'unatin *et al.* 2022). The potential of *W. confusa* isolated from longan (*D. longan*) fruit on EPS production has not been reported. Based on the results of this study, an increase in the pH of the media in alkaline conditions and the presence of sucrose stimulated high EPS production. This study was expected to provide information if *W. confusa* K2 from longan (*D. longan*) fruit produced high EPS and can be considered if used for production of EPS on a larger scale.

Acknowledgements

Thanks to the Biochemistry and Organic Chemistry Laboratory of the State Islamic University of Maulana Malik Ibrahim Malang.

References

Adebayo-Tayo, B., Ishola, R., Oyewunmi T., 2018. Characterization, antioxidant and immunomodulatory potential on exopolysaccharide produced by wild type and mutant *Weissella confuse* strains. *Biotechnol Rep.* 19, e00271. https://doi.org/10.1016/j.btre.2018.e00271

Adesulu-Dahunsi, A.T., Sanni, A.I., Jeyaram, K., Ojediran, J.O., Ogunsakin, A.O., Banwo, K., 2018. Extracellular polysaccharide from *Weissella confusa* OF126: production, optimization, and characterization. *International Journal of Biological Macromolecules*. 111, 514-525. https://doi.org/10.1016/j.ijbiomac.2018.01.060

Angelin, J., Kavitha, M., 2020. Review: Exopolysaccharides from probiotic bacteria and their health potential. *International Journal of Biological Macromolecules.* 162, 853-865. https://doi.org/10.1016/j.ijbiomac.2020.06.190

Dubey, A.K., Jeevaratnam K., 2015. Structural characterization and functional evaluation of an exopolysaccharide produced by *Weissella confusa* AJ53, an isolate from fermented uttapam batter supplemented with *Piper betle* L. leaves. *Food Science and Biotechnology*. 24, 2117-2124. https://doi.org/10.1007/s10068-015-0281-y

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. *Anal Chem.* 28, 350-356. https://doi.org/10.1021/ac60111a017

Farinazzo, F.S., Valente, L.J., Almeida, M.B., Simionato, A.S., Fernandes, M.T.C., Mauro, C.S.I., Tomala, A.A.B., Garcia, S., 2019. Characterization and antioxidant activity of an exopolysaccharide produced by *Leuconostoc pseudomesenteroides* JF17 from juçara fruits (*Euterpe edulis* Martius). *Process Biochem.* 91, 141-148. https://doi.org/10.1016/j.procbio.2019.12.005

Feng, F., Zhou, Q., Yang, Y., Zhao, F., Du, R., Han, Y., Xiao, H., Zhou, Z., 2018. Characterization of highly branched dextran produced by *Leuconostoc citreum* B-2 from pineapple fermented product. *Intl J Biol Macromol.* 113, 45-50. https://doi.org/10.1016/j.ijbiomac.2018.02.119

Holt, J.G., Krieg, N.R., Sneath, P.H.A., Staley, J.T., Williams, S.T., 1994. Bergey's manual® of determinative bacteriology, Ninth ed. Williams & Wilkins, Maryland.

Ju, Y., Shan, K., Liu, W., Xi, C., Zhang, Y., Wang, W., Wang, C., Cao, R., Zhu, W., Wang, H., Zhao, Y., Hao, L., 2022. Effect of different initial fermentation pH on exopolysaccharides produced by *Pseudoalteromonas agarivorans* Hao 2018 and identification of key genes involved in exopolysaccharide synthesis via transcriptome analysis. *Marine Drugs.* 20, 89. https://doi.org/10.3390/md20020089

Kavitake D., Devi P.B., Shetty P.H., 2020. Overview of exopolysaccharides produced by Weissella genus-a review. *International Journal of Biological Macromolecules*. 164, 2964-2973. https://doi.org/10.1016/j.ijbiomac.2020.08.185

Lakra, A.K., Domli, L., Tilwani, Younus, M., Arul V., 2020. Physicochemical and functional characterization of mannan exopolsaccharide from *Weissella confusa* MD1 with bioactivities. *International Journal of Biological Macromolecules*. 143, 797-805. https://doi.org/10.1016/j.ijbiomac.2019.09.139

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the folin phenol reagent. *J Biol Chem.* 193, 265- 275. https://doi.org/10.1016/S0021-9258(19)52451-6

Ma'unatin, A., Harijono, H., Zubaidah, E., Rifa'i, M., 2022. Dextran production using *Leuconostoc mesenteroides* strains isolated from *Borassus flabellifer* sap. *Biodiversitas*. 23, 1154-1158. https://doi.org/10.13057/biodiv/d230260

Netrusov A.I., Liyaskina E.V., Kurgaeva I.V., Liyaskina A.U., Yang G., Revin V.V., 2023. Exopolysaccharides producing bacteria: a review. *Microorganisms.* 11, 1541. https://doi.org/10.3390/microorganisms11061541

Nguyen, P.T., Nguyen, T.T., Bui, D.C., Hong, P.T., Hoang, Q.K., Nguyen, H.T., 2020. Exopolysaccharide production by lactic acid bacteria: The manipulation of environmental stresses for industrial applications. *AIMS Microbiology.* 6, 451-469. https://doi.org/10.3934/microbiol.2020027

Nguyen, P.T., Nguyen, T.T., Vo, T.N.T., Nguyen, T.T.X., Hoang, Q.K., Nguyen, H.T., 2021. Response of *Lactobacillus plantarum* VAL6 to challenges of pH and sodium chloride stresses. *Scientific Reports.* 11, 1-9. https://doi.org/10.1038/s41598-020-80634-1

Prete R., Alam M.K., Perpetuini G., Perla C., Pittia P., Aldo Corsetti, A. 2021. Lactic acid bacteria exopolysaccharides producers: a sustainable tool for functional foods. *Foods.* 10, 1653. https://doi.org/10.3390/foods10071653

Patel, S., Majumder, A., Goyal, A., 2012. Potentials of exopolysaccharides from lactic acid bacteria. *Indian Journal of Microbiology.* 52, 3-12. https://doi.org/10.1007/s12088-011-0148-8

Polak-Berecka, M., Was'ko, A., Paduch, R., Skrzypek, T., Sroka-Bartnicka, A., 2014. The effect of cell surface components on adhesion ability of *Lactobacillus rhamnosus. Antonie van Leeuwenhoek.* 106, 751-762. https://doi.org/10.1007/s10482-014-0245-x

Saadat, Y.R., Khosroushahi, A.Y., Gargari, B.P., 2019. A comprehensive review of anticancer, immunomodulatory and health beneficial effects of the lactic acid bacteria exopolysaccharides. *Carbohydr Polym.* 217, 79-89. https://doi.org/10.1016/j.carbpol.2019.04.025

Sanhueza, E., Paredes-Osses, E., González, C.L., García, A., 2015. Effect of pH in the survival of *Lactobacillus salivarius* strain UCO_979C wild type and the pH acid acclimated variant. *Electronic Journal of Biotechnology.* 18, 343-346. https://doi.org/10.1016/j.ejbt.2015.06.005

Saravanan, C., Shetty, P.K.H., 2016. Isolation and characterization of exopolysaccharide from *Leuconostoc lactis* KC117496 isolated from idli batter. *Int. J. Biol. Macromol.* 90, 100-106. https://doi.org/10.1016/j.ijbiomac.2015.02.007

Sharma, K., Sharma, N., Handa, S., Pathania, S., 2020. Purification and characterization of novel exopolysaccharides produced from *Lactobacillus paraplantarum* KM1 isolated from human milk and its cytotoxicity. *Journal Of Genetic Engineering and Biotechnology*. 18, 1-10. https://doi.org/10.1186/s43141-020-00063-5

Slížová, M., Nemcova, R., Mad'ar, M., Hadryová, J., Gancarčíková, S., Popper, M., Pistl, J., 2015. Analysis of biofilm formation by intestinal *Lactobacilli. Can J Microbiol.* 61, 1-10. https://doi.org/10.1139/cjm-2015-0007

Tayuan, C., Tannock, G.W., Rodtong, S., 2011. Growth and exopolysaccharide production by *Weissella* sp. from low-cost substitutes for sucrose. *Afr. J. Microbiol Res*. 5, 3693-3710. https://doi.org/10.5897/AJMR11.486

Tingirikari J.M., Kothari, D., Goyal, A., 2014. Superior prebiotic and physicochemical properties of novel dextran from *Weissella cibaria* JAG8 for potential food applications. *Food Funct.* 5, 2324-2330. https://doi.org/10.1039/C4FO00319E

Wang, Y., Li, C., Liu, P., Ahmed, Z., Xiao, P., Bai, X., 2010. Physical characterization of exopolysaccharide produced by *Lactobacillus plantarum* KF5 isolated from Tibet kefir, Carbohydr. *Polym.* 82, 895-903. https://doi.org/10.1016/j.carbpol.2010.06.013

Wang, J., Zhao, X., Yang, Y., Zhao, A., Yang, Z., 2015. characterization and bioactivities of an exopolysaccharide produced by *Lactobacillus plantarum* YW32. *International journal of biological macromolecules*. 74, 119-126. https://doi.org/10.1016/j.ijbiomac.2014.12.006

Zhou, Q., Feng, F., Yang, Y., Zhao, F., Du, R., Zhou, Z., Han, Y., 2017. Characterization of a dextran produced by *Leuconostoc pseudomesenteroides* XG5 from homemade wine. *International Journal of Biological Macromolecules.* 107, 2234-2241. https://doi.org/10.1016/j.ijbiomac.2017.10.098