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Production, Characterization, and Antioxidant Activities of an Exopolysaccharide Extracted from Spent Media Wastewater after *Leuconostoc mesenteroides* WiKim32 Fermentation

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ABSTRACT: Bacterial exopolysaccharides (EPSs) are important alternatives to plant polysaccharides in fermented products and exhibit antioxidant activity, which is particularly desirable for functional foods. This study evaluated the use of spent media wastewater (SMW) derived from kimchi fermentation for the production of an EPS and analyzed the characterization and antioxidant activity of the resulting EPS. The EPS concentration and conversion yields of sequential purification were 7.7−9.0 g/L and 38.6−45.1%, respectively. Fourier transform infrared spectra and NMR spectra indicated that the EPS was a linear glucan with α-(1 → 6) linkages. The EPS also exhibited thermal tolerance to high temperatures. *In vitro* antioxidant activity analyses indicated the scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, thiobarbituric acid reactance (TBAR), and ferric ion reducing antioxidant power (FRAP) values of 71.6−79.1, 28.2−33.0%, and 0.04−0.05 mM $FeCl_3$, respectively. These results reveal that the EPS extracted from SMW has potential as a thermally tolerant, nontoxic, and natural antioxidant for industrial applications.

1. INTRODUCTION

Lactic acid bacteria (LAB) are food-grade microorganisms that are widely applied in a variety of fermentation industries.[1] There is growing interest in the industrial applications of LAB, for example, as an important starter culture; however, the quality of fermented foods depends on the specific LAB species involved in the process.[2] The demand for fermented products has increased significantly in recent years, which has led to an increase in the generation of spent media wastewater (SMW) from fermentation industries. Industrial waste is typically disposed off into the environment, causing substantial environmental problems. However, SMW is rich in polysaccharides that can be utilized in the sustainable production of commercially valuable products.[3]

LAB can produce natural polysaccharides with an enormous structural diversity. Bacterial exopolysaccharides (EPSs), which are synthesized or secreted into the extracellular matrix of LAB, are attached to the cell surface or released into the surrounding environment. An EPS comprises several monosaccharides linked by glycosidic bonds that blend into various structural compositions.[4] EPSs have attracted attention in many scientific fields, with a particular focus on the optimization of production processes and functional properties. EPSs possess enormous functional applications in cosmetology, food additives, and pharmacology. The structure and character of bacterial EPSs mainly depend on the strains and fermentation conditions. Compared to other natural polysaccharides, EPSs require shorter production time and a much simpler extraction process. Recent studies have examined the potential health benefits of EPSs and their industrial applications.[5] In the food industry, EPSs are regarded as alternatives to plant polysaccharides because they contribute to the texture modification, taste recognition, and stability of fermented foods. Furthermore, EPSs exhibit biological activities, especially antioxidant activity, which is desirable for functional foods and other industries.[6]

Reactive oxygen species (ROS) play important roles in maintaining the immune system and the redox balance, such as apoptosis, cell signaling, and ion transportation.[7] However, excessive ROS can cause damage to the body through a variety of pathological effects, such as cancer, diabetes, and atherosclerosis.[8,9] Lipid oxidation is an important reaction in

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both biological and food systems and is considered a deteriorative reaction in food containing lipids.[10] Many studies have attempted to prevent lipid oxidation in food products, and adding antioxidants during food processing is one of the most effective strategies.[11−13] Antioxidants can prevent lipid oxidation through various mechanisms, including scavenging free radicals, decomposing lipid peroxides, and preventing the formation of peroxides.[14] Although synthetic antioxidants, i.e., butylated hydroxyanisole (BHT) and butylated hydroxytoluene (BHA), have a strong radical-scavenging ability, harmful effects and toxicity limit their application in the food industry.[15] Therefore, it is of substantial interest to find natural, harmless, and nontoxic antioxidants instead of chemical antioxidants.

Leuconostoc mesenteroides is the dominant LAB present during early kimchi fermentation and has been commercialized for kimchi starters because of its beneficial effects on sensory characteristics.[16] After cultivation, the cells are harvested for the kimchi starter and the fermentation liquid is discarded as industrial waste. In this study, an EPS is extracted and purified from the fermentation liquid produced after kimchi starter cultivation, and its physiochemical properties are characterized. Moreover, the production rate and conversion yield of EPS are calculated based on the initial fermentation media. Furthermore, the scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, ferric ion reducing antioxidant power (FRAP), and thiobarbituric acid reactance (TBAR) are analyzed to determine the *in vitro* antioxidant activity of EPS.

2. RESULTS AND DISCUSSION

2.1. Production and Purification of EPS. Glucose and sucrose are commonly used as carbon sources for the cultivation and production of bacterial EPSs.[17] Considering that the fermentation media account for 30% of the total cost of EPS production, research is focused on identifying cheaper substrates such as agricultural waste, industrial waste, and byproducts.[18] However, low-cost waste substrates exhibit negative effects—i.e., different polymers or unexpected byproducts may be synthesized owing to the presence of contaminants and the different metabolic pathways that might be followed by different nutrient compositions.[1] SMW, which is discarded after the cultivation of the kimchi starter, does not require an additional carbon source for EPS production.

In this study, the production of EPS and the conversion of sucrose to EPS were calculated according to the sucrose concentration (20 g/L) in the fermentation media. The crude exopolysaccharide (cEPS) may have contained byproducts such as proteins or peptides during fermentation. Since these impurities were removed through purification, the concentration of EPS decreased from 9.0 g/L in cEPS to 7.7g/L in purified exopolysaccharide (pEPS). The conversion yields of cEPS and pEPS were 45.1 and 38.6%, respectively (Table 1).

Several previous studies have examined the optimal conditions to produce EPSs; the EPS concentration and sucrose−EPS conversion yield obtained in this study were either similar to or greater than those observed in previous studies. For example, EPS production from molasses medium (17.5% sucrose) produced 5.4−5.5 g dextran with a conversion yield of approximately 31%.[19] Another study reported that the fermentation of *L. mesenteroides* after optimization produced approximately 4.9 g/L of EPS with a conversion yield of 48.9%.[20]

Table 1. Exopolysaccharide (EPS) Production from Spent Media Wastewater (SMW)[a]

	concentration (g/L)[b]	conversion yield[c] (%, w/w)
cEPS	9.0 ± 0.7	45.1 ± 3.4
pEPS	7.7 ± 0.9	38.6 ± 4.4

[a]Abbreviations: cEPS, crude EPS; pEPS, purified EPS. [b]Values represent the average of three replicates. [c]Conversion yield was calculated based on the sucrose concentration in the fermentation media.

2.2. Chemical Composition and Structural Properties. EPSs produced by LAB can be classified into homosaccharides and heterosaccharides.[21] Homopolysaccharides are composed of one type of carbohydrate such as fructose or glucose, whereas heterosaccharides contain two or more monosaccharides, mainly glucose, galactose, fructose, and mannose in different ratios (Table 2). *L. mesenteroides* is used to produce a glucose polymer known as dextran or alternan.[20]

The carbohydrate of the EPS produced from *L. mesenteroides* WiKim32 contained glucose (Figure 1). No other monosugar was detected (Figure 1A,C). With purification, the glucose content of EPS increased from 59.6% (cEPS) (Figure 1B) to 75.1% (pEPS) (Figure 1D). EPS carbohydrates were affected differently by the purification. Moreover, the protein content decreased from 13.2 to 8.5%, suggesting that the EPS may be a protein-bound polysaccharide.

This is because the EPS is trapped within the protein matrix and tightly bound to the cell when polysaccharides are layered on the bacterial surface with glycoproteins.[27] In previous studies, the EPS separated from *L. mesenteroides* DRP105 contained 1.85% protein, indicating that it may be a proteoglycan.[28]

As shown via sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), the protein concentrations of whole protein extracts of *L. mesenteroides* WiKim32 differed between cEPS and pEPS (Figure 2). Several intense cEPS protein bands were observed when compared to those of pEPS; however, these were not observed in dextran. The Fourier transform infrared (FTIR) spectrum of EPS is presented in Figure 3A. UV spectra of pEPS showed a peak at 260 nm, supporting the presence of proteins after the purification process (Figure S1 in the Supporting Information). The band at 3305 cm^{-1} corresponded to the hydroxyl (OH) group,[29] and the band at 2922 cm^{-1} was attributed to asymmetrical and symmetrical C−H.[30] The absorption peak at 1658 cm^{-1} was a polysaccharide and attributed to C═O stretching.[31] The intense peak at 1343 cm^{-1} was severed carboxyl or carboxylate groups.[32] The peak at 1011 cm^{-1} confirmed that the EPS contained α-glycosidic linkages,[33] and the absorption at 917 and 843 cm^{-1} indicated a glucosyl residue with α-pyranose.[34] The absence of a band at 890 cm^{-1} revealed the absence of a β-glycosidic linkage in the EPS.[35] The FTIR spectra of the EPS from *L. mesenteroides* WiKim32 exhibited similar properties to those from *L. pseudomesenteroides*[17] and *L. citreum*.[36] FTIR spectral analysis of *L. mesenteroides* WiKim32 EPS indicated the α-configuration and α-glucosidic polysaccharide.

NMR spectroscopy is a commonly used tool in the structural analysis of complex compounds. The ^{13}C NMR spectrum of EPS, which included ring carbons (50−85 ppm) and anomeric carbon regions (95−110 ppm), is presented in Figure 3B. The anomeric carbon peak at δ 100.6 ppm was attributed to the C1

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Table 2. Monosaccharide Composition of Microbial Exopolysaccharides (EPSs) and Related Antioxidant Activities[a]

EPS-producing microorganisms	monosaccharides	antioxidant activity	references
Enterococcus faecium K1	mannose, glucose, galactose	higher scavenging of hydroxyl and DPPH	Bhat and Bajaj[22]
Weissella cibaria SJ14	mannose, glucose, galactose, arabinose, xylose, rhamnose	higher scavenging of DPPH	Zhu et al.[23]
Weissella confuse	galactose, mannose, glucose, fructose, rhamnose, arabinose, xylose, ribose	higher scavenging of DPPH, H_2O_2 radicals	Adebayo-tayo et al.[24]
Lactobacillus delbrueckii ssp.	galactose, glucose	higher scavenging of superoxide, hydroxyl, DPPH radicals	Tang et al.[25]
Lactobacillus plantarum KX041	arabinose, mannose, glucose, galactose	higher scavenging of ABTS, DPPH	Wang et al.[15]
L. plantarum ZDY2013	galactose, xylose	higher scavenging of hydroxyl radical	Zhang et al.[26]
L. mesenteroides WiKim32	glucose	higher scavenging of ABTS, DPPH, TBARS	this study

[a]Abbreviations: DPPH, 1,1-diphenyl-2-picrylhydrazyl; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); and TBARS, thiobarbituric acid reactive substances.

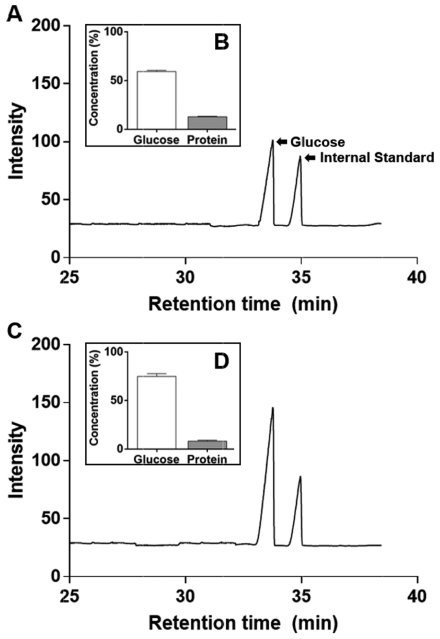

Figure 1. Chromatogram and chemical composition of crude exopolysaccharide (cEPS) (A, B) and purified exopolysaccharide (pEPS) (C, D).

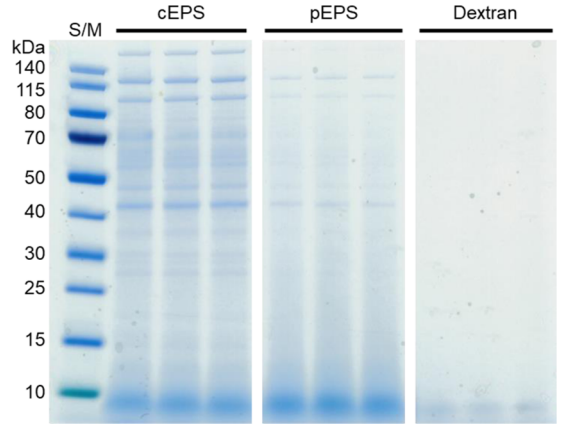

Figure 2. Protein weight distribution of the exopolysaccharide (EPS) on SDS-PAGE (S/M: protein ladder).

α-glucopyranosyl repeating unit. The clusters of resonances around the peaks at δ 74.3, δ 76.3, δ 73.0, and δ 72.4 ppm were attributed to C-2, C-3, C-4, and C-5, respectively. No additional peaks appeared within the region of δ 78−85 ppm, indicating the absence of branched linkages. The absorption peak at δ 68.4 ppm was attributed to C-6 of glucose in the α-(1 → 6) linkage polymer.

These results indicate that the EPS from *L. mesenteroides* WiKim32 is a highly linear dextran with α-(1 → 6) glycosidic linkages (Figure S2 in the Supporting Information) and without branch linkages according to FTIR and NMR results. These structural properties were similar to those of the EPS from *L. pseudomesenteroides*,[17] *L. pseudomesenteroides* YF32,[36] and *L. mesenteroides* TDS2-19.[37]

2.3. Transmission Electron Microscopy (TEM). Transmission electron microscopy (TEM) is an essential tool for investigating nanostructural and spatial aspects in biology. *L. mesenteroides* WiKim32 cells exhibited well-defined cytoplasmic contents and an intact cell wall (Figure 4). An EPS thickness of 37.2 ± 3.4 nm was observed, covering the cell surfaces (Figure 4B). Generally, the cell walls of LAB are formed by four structural models: (i) cell wall made of peptidoglycan, lipoteichoic acids (LTA), and polysaccharides; (ii) EPS attached to the outer surface; (iii) peptidoglycan-containing layer enveloped by the surface layer protein; and (iv) polymer layer cross-linked with the surface layer protein.[2] The cell wall structure of *L. mesenteroides* WiKim32 according to these models is shown in Figure 4C. The functional properties of EPSs have attracted increasing interest in health-related applications, for example, as natural antioxidants.[38,39]

2.4. Thermodynamic Behavior. Thermogravimetric analysis (TGA) of EPS was conducted for weight loss in the temperature range 30−550 °C (Figure 5). The results showed that the EPS degraded in three stages. The first stage corresponds to the moisture drying region (at 30−147 °C) with a 12% weight loss. This behavior is similar to the decomposition of the EPS from *L. plantarum* KF5.[40] The EPS remained stable and exhibited thermal tolerance when the temperature increased from 147 to 260 °C. The final stage was 260−320 °C, during which a major loss of mass (42%) occurred due to the depolymerization of EPS. Based on the TGA results, the thermal behavior of dextran was similar to that of EPS, which was due to the similar structure and composition of EPS, derived from *L. mesenteroides* WiKim32, and commercial dextran (data not shown). These thermody-

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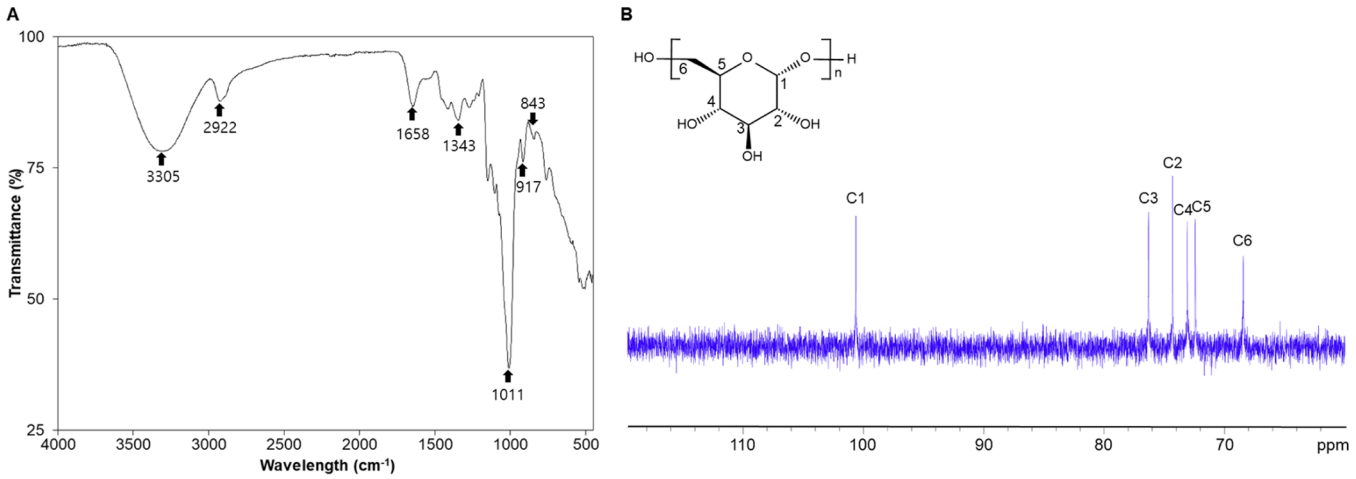

Figure 3. Structural analysis. (A) Fourier transform infrared (FTIR) spectrum and (B) ^{13}C NMR spectrum of the purified exopolysaccharide (EPS) from *L. mesenteroides* WiKim32.

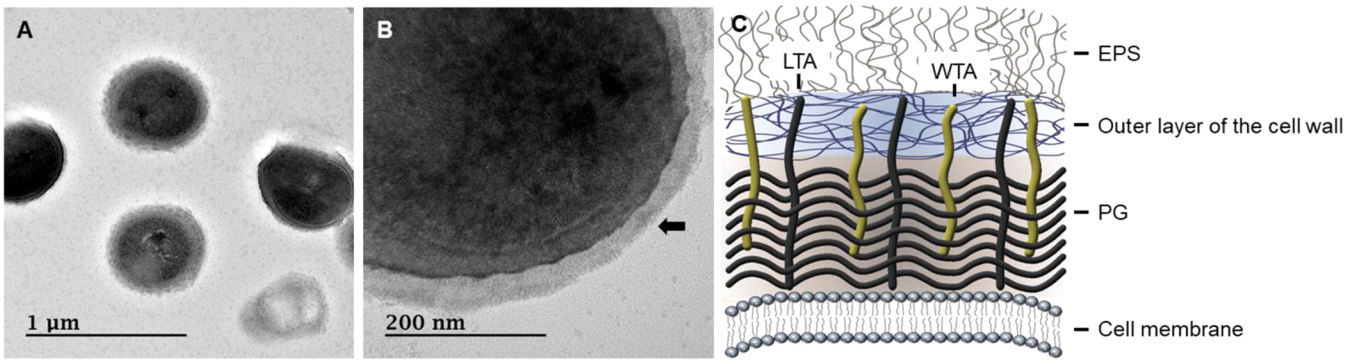

Figure 4. Transmission electron microscopy (TEM) images of *L. mesenteroides* WiKim32. (A) Bacterial cells after cultivation. (B) Layer at the surface of the cell wall represents the exopolysaccharide (EPS) (black arrow). (C) Structural models of the *L. mesenteroides* WiKim32 cell wall. The bacterial cell envelops the peptidoglycan layer (PG), wall teichoic acids (WTA), lipoteichoic acids (LTA), and the outer layer of the cell wall. The EPS is attached to the cell wall. Scale bars are 1 μm (left) and 200 nm (middle).

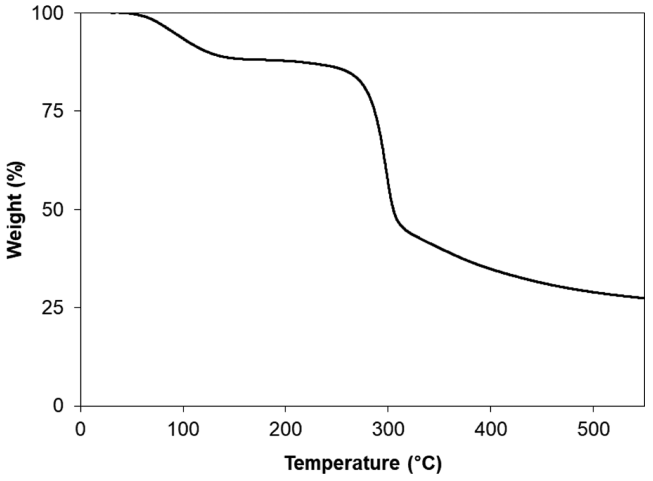

Figure 5. Thermogravimetric analysis (TGA) curve of the purified exopolysaccharide (EPS) from spent media wastewater of *L. mesenteroides* WiKim32.

namic results indicate that EPS has a relatively high degradation temperature; this thermal property can be applied in various industries to improve the thermal tolerance of products.[41]

2.5. Antioxidant Activity. *2.5.1. DPPH Radical-Scavenging Activity.* DPPH is a commonly used compound in the assay for evaluating the free-radical-scavenging capacities of antioxidants. During the assay, the antioxidants reduced the stable DPPH radical and the nonradical form of DPPH-H. The effect of antioxidants on DPPH is closely related to its hydrogen donation ability. The results indicate that cEPS and pEPS influence free-radical scavenging (Figure 6A). The DPPH radical-scavenging activity differed for the materials tested (F = 159.0; df = 2, 6; P < 0.001).

The radical-scavenging activities of cEPS and pEPS were 79.1 and 71.6% at 10 mg/mL, respectively, indicating that the scavenging activity of cEPS was higher than that of pEPS. This is because other antioxidant components, such as proteins, amino acids, and microelements, are present in the cEPS.[42] These components may exhibit some interactions and may have synergistic effects on the antioxidant activity.[43]

2.5.2. TBAR Assay. Lipid peroxidation may occur during oxidative damage to cell structures and the toxicity process, leading to cell death. Oxidative alteration of polyunsaturated fatty acids generates multiple degradation products by lipid peroxidation.[44] In this study, the $FeCl_2$−H_2O_2 system was adopted to induce lipid peroxidation. The lipid peroxidation inhibition differed for each material (F = 734.9; df = 2, 6; P < 0.001) (Figure 6B). The inhibition effects of BHT, cEPS, and pEPS were 76.6, 33, and 28.2%, respectively, at a concentration

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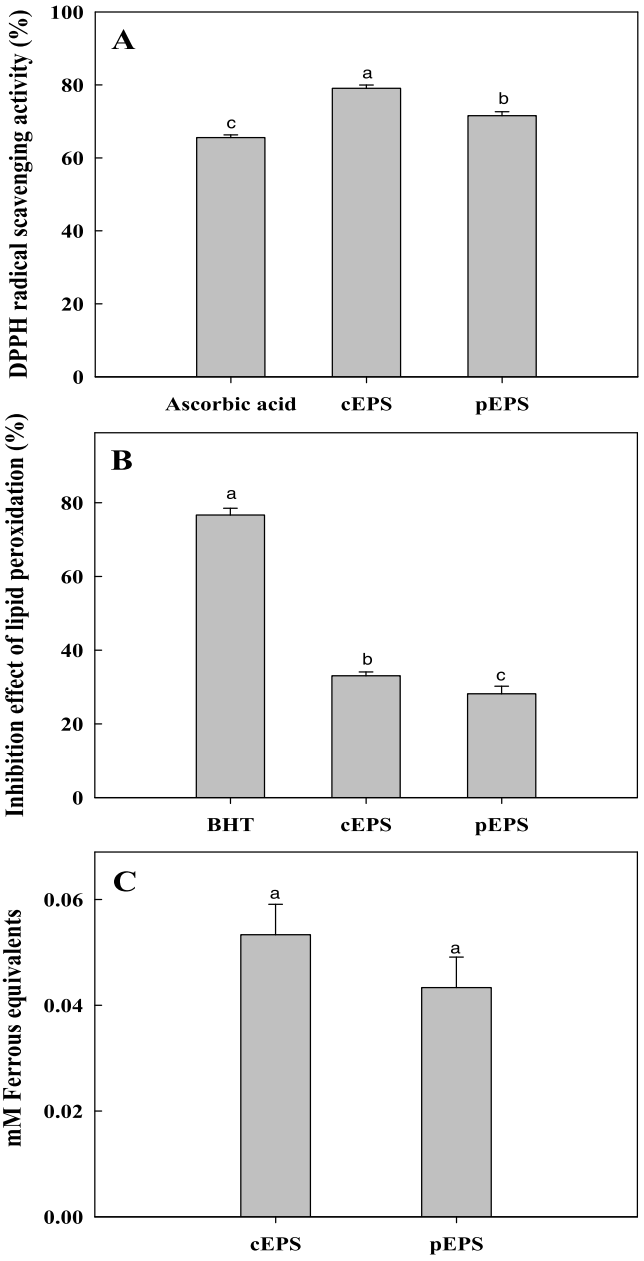

Figure 6. Antioxidant activity of cEPS and pEPS: (A) radical-scavenging capacity of cEPS and pEPS, (B) inhibition effect of lipid peroxidation, and (C) ferrous chelating ability.

of 2.5 mg/mL (Figure 5B). These results indicate that EPS has moderate inhibitory effects on lipid peroxidation compared with BHT. The inhibition of lipid peroxidation may be attributed to their scavenging abilities on hydroxyl radicals and the H_2O_2 produced by the $FeCl_2$−H_2O_2 reaction.[45] Another study reported that polysaccharides exhibit inhibitory effects on lipid peroxidation because of their metal ion chelating properties.[46]

2.5.3. FRAP Assay. The FRAP assay is a simple and direct method of measuring the antioxidative ability of a substance required to directly reduce an oxidant. The reducing potential of antioxidants is related to the ability of electron donation to break the free-radical chain reactions.[47] In this study, the FRAP assay was conducted to evaluate the antioxidant power of EPS.

Based on the calibration curve of $FeCl_3$, the FRAP values for cEPS and pEPS were calculated as 0.05 mM $FeCl_3$ and 0.04 mM $FeCl_3$, respectively (Figure 5C). This indicates that EPS may act as an electron donor and react with free radicals, which can terminate the free-radical chain reaction for stable products. EPS also showed scavenging abilities on hydroxyl radicals and metal ion chelating activities. Therefore, the lipid peroxidation inhibition effects of EPS may be attributed to the Fe^{2+} chelating ability of polysaccharides.[44]

2.6. Overall Mass Balance. An overall mass balance diagram describing the bacterial fermentation, harvest, and extraction of EPS is shown in Figure 7. *L. mesenteroides*

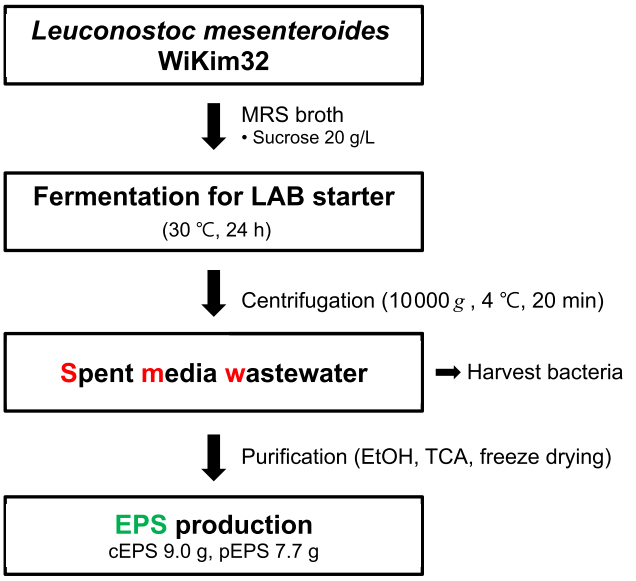

Figure 7. Overall mass balance.

WiKim32 was fermented for the kimchi starter at 30 °C for 24 h. The bacterial cells were harvested, and the supernatant was collected by centrifugation. Through extraction and purification using ethanol and TCA, 9.0 g of cEPS and 7.7 g of pEPS were obtained with conversion yields of 45.1 and 38.6% per 20 g sucrose, respectively. These results suggest that SMW is a potential resource for EPS production.

3. CONCLUSIONS

This study revealed the potential antioxidant activities of an EPS obtained from SMW derived from the kimchi fermentation process. The EPS concentration and sucrose−EPS conversion yield were similar to or greater than the optimized values. The obtained EPS exhibited structural properties and thermal tolerance similar to dextran. In addition, the functional and antioxidant properties of EPS were examined, revealing DPPH radical-scavenging activity, a lipid peroxidation inhibition effect, and ferrous chelating ability. Further pharmacological research on EPS is required to exploit it as a natural alternative to commercial antioxidants. Moreover, SMW raises the potential for more economic EPS production.

4. MATERIALS AND METHODS

4.1. EPS Production and Isolation. *4.1.1. Microorganism Cultivation.* *L. mesenteroides* WiKim32 (KFCC11639P), developed as a kimchi fermentation starter, was used in this study. The bacteria were cultured in an MRS medium (peptone 10 g/L, beef extract 10 g/L, yeast extract 5 g/L,

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glucose 20 g/L, Tween 80 1 g/L, ammonium citrate 2 g/L, sodium acetate 5 g/L, magnesium sulfate 0.1 g/L, manganese sulfate 0.05 g/L, and dipotassium phosphate 2 g/L) at 30 °C for 24 h. Then, the cultures were stored at −80 °C (MDF4V; Panasonic, Tokyo, Japan) in the presence of 20% glycerol as a cryoprotectant until further use. To produce the kimchi starter, the freeze-dried cells were cultured in 10 L of modified MRS broth (glucose was replaced by sucrose 20 g/L) and harvested when they reached the midexponential phase of growth (OD 600 nm of 0.6). After cultivation, the cells were collected (10 000*g* for 20 min at 4 °C) and prepared to produce the kimchi fermentation starter.

4.1.2. Purification of EPS. The EPS was isolated and purified following the method of Adesulu-Dahunsi et al.[11] with slight modifications. After cell cultivation and collection, SMW was heated at 100 °C for 10 min to inactivate the enzymes, and the debris was removed by centrifugation (10 000*g* for 20 min at 4 °C). Three volumes of 95% (v/v) cold ethanol were added to the supernatant and stored at −4 °C overnight and then centrifuged (10 000*g* for 20 min at 4 °C). Crude EPS (cEPS) was obtained after ethanol precipitation and lyophilization. To remove the protein, cEPS was dissolved in distilled water and deproteinized using 4% (v/v) trichloroacetic acid (TCA). cEPS was reprecipitated with three volumes of 95% (v/v) cold ethanol and dissolved in distilled water. Finally, dialysis was conducted twice daily with distilled water for 2 days at 4 °C using dialysis cassettes (Slide-A-Lyzer 10K MWCO, Thermo Scientific, Waltham, MA) and then lyophilized. The final residual was termed pure EPS (pEPS).

4.2. Analytical Methodology. *4.2.1. Monosugar Composition and Protein.* Monosaccharide compositions were analyzed for their neutral sugar content using gas chromatography (GC). The neutral sugar composition was measured with alditol acetates containing *myo*-inositol as an internal standard. Samples were analyzed with GC (7890A, Agilent Technology, Palo Alto, CA) using a DB-225 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness; J&W, Rancho Cordova, CA). The chromatograph was operated with He at an injector temperature of 220 °C and a flame ionization detector at 250 °C; the oven temperature programming was set to 100 °C for 1.5 min, which was then increased by 5 °C/min up to 220 °C.[48] The protein concentration was measured using the Lowry method, with bovine serum albumin (BSA) as a protein standard. Proteins in the EPS were separated on 4−12% Bis−Tris NuPage gels (Invitrogen, Carlsbad, CA) in a Mini-Gel tank (Thermo Fisher Scientific, Waltham, MA) at 220 V for 30 min. Gels were stained with SimplyBlue SafeStain (Thermo Fisher Scientific). Based on sucrose and EPS concentrations, the conversion yields were calculated using the following formula

$$\text{conversion yield (\%)} = C_{EPS}/C_{sucrose} \times 100$$

where $C_{sucrose}$ is the amount of sucrose in the fermentation media and C_{EPS} is the amount of cEPS or pEPS. Ultraviolet−visible (UV−vis) spectroscopy analyses were conducted using a Nanophotometer UV−vis spectrophotometer NP80 (Implen, Munchen, Germany). The sample was prepared in distilled water (1 mg/mL) for UV measurements in a wavelength range of 200−900 nm.

4.2.2. Structural Characterization. The morphologies of the bacterial cells were examined using a transmission electron microscope (JEM-1400; JEOL, Tokyo, Japan). Samples were fixed in 2% (v/v) glutaraldehyde (Merck, Darmstadt, Germany), dehydrated to 30, 50, and 70% (v/v) twice in absolute ethanol, and then sectioned using an ultramicrotome with a diamond knife.[2] The EPS thickness was determined using Gatan digital micrograph software. The average diameter was measured at different regions of each cell for 16 bacteria. A Fourier transform infrared (FTIR) spectrum of the lyophilized sample was obtained with the ATR technique using a PerkinElmer Spectrum 400 (PerkinElmer, Inc., Shelton, CT). The spectrum was scanned in the range of 4000−400 cm^{-1} using four scans with a resolution of 4 cm^{-1}. NMR spectra were recorded on a Varian Unity Inova 500 MHz spectrometer (Varian NMR Systems, Palo Alto, CA), operating at 125 MHz for ^{13}C NMR. The data were determined and processed using TopSpin 4.0 software. Thermogravimetric analysis (TGA) was performed using Mettler Toledo equipment (Mettler Toledo, Schwarzenbach, Switzerland). To determine the carbonization temperature, the samples were analyzed in the range of 30−550 °C under a nitrogen atmosphere.

4.3. Antioxidant Activity Assay. *4.3.1. DPPH Radical-Scavenging Assay.* The scavenging activity on DPPH free radicals was assayed according to the method by Shimada et al.[49] with slight modifications. Samples were dissolved in distilled water to a concentration of 1% (10 mg/mL). One milliliter of the sample was added to the same volume of 0.2 mM DPPH solution. The reaction mixture was mixed vigorously and incubated in the dark at 25 °C for 30 min. The absorbance was measured at 517 nm with ascorbic acid as the reference (50 μg/mL). The scavenging activity was defined as follows

$$\text{DPPH radical scavenging activity (\%)} = [1 - (A_{sample} - A_{blank})/A_{blank}] \times 100$$

4.3.2. Thiobarbituric Acid Reactive Substances Assay. The inhibition rate of lipid peroxidation was evaluated using the thiobarbituric acid (TBA) method from Chen et al.,[50] which is based on estimating the inhibition of linoleic acid peroxidation by samples. Briefly, 4% TCA, 0.8% TBA, and 0.4% ascorbic acid solutions were added to the samples and incubated at 100 °C for 10 min. The absorbance of the supernatant was measured at 532 nm with BHT as the reference (3 mM). The inhibition rate of lipid peroxidation was calculated using the following formula

$$\text{inhibition rate of lipid peroxidation (\%)} = (1 - A_{sample}/A_{blank}) \times 100$$

4.3.3. Ferric Reducing Antioxidant Power Assay. A ferric reducing antioxidant power (FRAP) assay was performed using a commercially available assay kit (Abcam, ab234626, Caliph, MI). Briefly, the total volume of the reaction mixture was 200 μL, and the reaction mixture comprised 152 μL of FRAP assay buffer, 19 μL of FRAP probe, 19 μL of $FeCl_3$ solution, and 10 μL of the sample. The microplates containing the reaction mixtures were kept in the dark at 37 °C for 60 min. The increase in absorbance of the mixtures was measured at 594 nm using a microplate reader. The antioxidant capacity was calculated using a ferrous iron standard curve, and the results were expressed as Fe^{2+} equivalents (μM).

4.4. Statistical Analysis. Data are presented as the mean of three independent experiments. Data were analyzed using IBM SPSS Statistics for Windows, Version 19 (IBM Corp., Armonk, NY). Analysis of variance followed by Tukey's

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honestly significant difference test was used to determine significant differences between treatments at $P < 0.05$.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c06095.

UV spectra of EPS in the range 200−900 nm (Figure S1); and Fourier transform infrared spectrum of commercial dextran (Figure S2) (PDF)

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I.S.C. and H.W.P. designed the study. I.S.C., S.H.K., M.E.L., H.M.K., J.E.Y., S.-G.J., and J.-C.K. performed the experiments. K.H.L., J.Y.C., and J.-C.K. contributed to data interpretation. I.S.C. and H.W.P. wrote the main manuscript. All authors read, reviewed, and approved the final manuscript.

Notes

The authors declare no competing financial interest.

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