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Characterization and antioxidant activity of an acidic exopolysaccharide from *Lactobacillus plantarum* JLAU103

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An acidic exopolysaccharide (EPS) designated as EPS103 was isolated, purified and characterized from *Lactobacillus plantarum* JLAU103. EPS103 had a relatively lower molecular weight of 12.4 KDa and was consisted of arabinose, rhamnose, fucose, xylose, mannose, fructose, galactose, and glucose in an approximate molar ratio of 4.05: 6.04: 6.29: 5.22: 1.47: 5.21: 2.24: 1.83. A specific spectrogram of acidic polysaccharide was obtained by FT-IR analysis, and both α- and β-type configurations were presented in EPS103 based on the nuclear magnetic resonance spectroscopy. Microstructural analysis of EPS103 demonstrated a smooth and glittering cube structure, and presence of many homogeneous rod-shaped lumps. Comprehensive study of *in vitro* antioxidant activity indicated that EPS103 possess strong scavenging abilities against hydroxyl, ABTS, and DPPH radicals with the maximum of 80.4%, 65.5%, and 60.5%, respectively, at 10 mg/mL concentration. Furthermore, EPS103 also showed strong ferrous ions chelating activity and oxygen radical absorbance capacity. These results together indicated that the EPS103 isolated from *L. plantarum* JLAU103 has great potential for use as a natural antioxidant or functional additive in foods industry.

[**Key words:** Exopolysaccharide; *Lactobacillus plantarum*; Purification; Antioxidant activity; Oxygen radical absorbance capacity]

Exopolysaccharide (EPS) represent a large group of high-molecular weight biopolymers that are produced by a wide range of microorganisms in natural ecological environments during the normal metabolic processes (1). EPS are either attached to the bacterial surface (capsular EPS) or released to the growth environment (ropy EPS) (2). Meanwhile, they can also be classified as homo- or hetero-polysaccharides according to whether they are composed of one or more types of sugar (3). Many lactic acid bacteria (LAB) are able to produce EPS that are known to possess the positive effects on the rheological, textural, functional and sensory properties of fermented products (4,5). For instance, the EPS have been used as thickening agent or stabilizer in low-fat cheese and yoghurt to improve textural and rheological characters (6,7). In addition to their technical properties, LAB EPS have been aroused widespread concern attribute to their various health-promoting potentials, such as antioxidant, immunoregulatory, antitumor, antibacterial, antidiabetic, hypocholesterolemic, and so on (8–12). Furthermore, recent researches revealed that the EPSs are important for LAB because of potential roles in stress resistance, adhesion, colonization, and host–bacteria interactions (13–15).

Natural antioxidants have received extensive attentions by researchers due to the increased number of diseases caused by the formation of free radicals and/or reactive oxygen species (ROS) (16). ROS is a natural byproduct of normal oxygen metabolism and plays an important role in cell signaling and homeostasis. It has been shown that excessive production of ROS in cells as a result of environmental stress may cause serious damage to DNA, RNA, and proteins, and may even lead to cell death (17). Although synthetic antioxidants are quite effective in slowing the oxidation process, their safety has recently been questioned (18). Therefore, it is greatly significant to explore and utilize natural antioxidants with high antioxidant activities and low cytotoxicities. In the last decade, lots of LAB with EPS-producing abilities were isolated from various fermented foods such as cheese, yoghurt, sausage, kefir, and sauerkraut (19). These LAB belong to the species of *Lactobacillus*, *Streptococcus*, *Pediococcus*, *Lactococcus*, and *Bifidobacterium*. *Lactobacillus plantarum* strains demonstrated versatility in the processing and preservation of fermented foods due to their special physicochemical and health-promoting benefits. Recently, some *L. plantarum* strains isolated from different indigenous fermented foods of China such as Tibet Kefir, Inner Mongolia fermented dairy tofu, and Paocai have been reported to produce EPS with different yields, characterization and biotechnological potential (20–22).

Hurood is a Chinese traditional fermented dairy food and produced and consumed primarily in Inner Mongolia of China, characterized by its soft texture, pleasant taste, and beneficial effects on human health including cholesterol lowering, antioxidative, and immune system-stimulating activities (23). It is rich in various LAB which are dominant microorganisms under the fermentation conditions (24). In our previous studies, we have screened some EPS-producing LAB strains from Hurood samples through the ropiness phenotype of the colony combined with EPS content determination. The select *L. plantarum* JLAU103 possessed an excellent ropy character and EPS-producing ability. Furthermore,

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this strain had good resistance at high concentrations of acid and salt, as well as strong *in vitro* antioxidant activity. Based on these, it is greatly significant to study the characterization and antioxidant activity of the EPS from *L. plantarum* JLAU103, which will be helpful for expanding its potential application in fermented food. Therefore, the present study was conducted to isolate and purify the EPS produced by *L. plantarum* JLAU103, partially characterize it, including determination of its molecular weight, monosaccharide composition, FT-IR and nuclear magnetic resonance (NMR) spectra, and microstructure, and to evaluate its *in vitro* antioxidant activity.

MATERIALS AND METHODS

Bacterial strains and growth media The EPS-producing strain *L. plantarum* JLAU103 previously isolated from Hurood in Inner Mongolia of China was maintained in de Man Rogosa Sharpe (MRS) medium at −80°C supplemented with 20% (v/v) glycerol. A semi-defined medium (SDM) was used for producing EPS (25). The composition per liter of the SDM is Bacto proteose peptone (Difco) (10 g), Bacto beef extract (Difco) (10 g), Bacto yeast extract (Difco) (5 g), D-sorbitol (Sigma) (20 g), ciprofloxacin (Sigma) (4 mg), sodium acetate (5 g), ammonium citrate (2 g), potassium phosphate (2 g), magnesium sulfate (0.1 g), magnesium sulfate (0.05 g), bromocresol purple (0.02 g) and Bacto agar (Difco) (15 g). The medium without ciprofloxacin was autoclaved for 15 min at 121°C, and cooled at 50°C. Ciprofloxacin was sterilized by filtration before being added to the cooled medium. The pH of the medium was adjusted to 6.0 ± 0.1.

Extraction and purification of EPS *L. plantarum* JLAU103 was incubated in SDM at 37°C for 24 h. Then the crude EPS was extracted and purified from the culture medium according to our previous methods (1). Briefly, 5 mL of crude EPS solution (20 mg/mL) was firstly separated with DEAE-cellulose column (2.6 cm × 40 cm) using deionized water, 0.2 and 0.5 M NaCl solution as eluent at a flow rate of 1 mL/min. Every 5 mL of elution was collected automatically and the carbohydrate content was determined by phenol–sulfuric acid method. Peak fractions containing polysaccharides were pooled, dialyzed, and lyophilized. Then the fraction was further purified on a Sepharose CL-6B gel column (2.5 cm × 50 cm) and eluted with 0.9 M NaCl solution at a flow rate of 0.5 mL/min. A purified fraction was obtained for the further work.

Chemical composition of EPS Total sugar content was determined by phenol-sulfuric acid method using glucose as standard (26). Protein content was determined by the method of binding of Coomassie Brilliant Blue G-250 to protein, using bovine serum albumin as a standard (27). Moisture content was estimated according to the method of Vijayendra et al. (28). Uronic acid content was determined by the Dische method, using glucuronic acid as standard (29). Sulfuric ester content was estimated according to the method reported by Therho and Hartiala (30).

Molecular weight determination The molecular weight was determined by using high performance Size exclusion chromatography (HPSEC) equipped with a TSK gel GMPWXL column (7.8 mm × 30 cm) and a refractive index detector (RID). The column was eluted with 0.1 M Na_2NO_3 + 0.06%NaN_3 solution at a flow rate of 0.6 mL/min at 35°C. Injection volume of sample was 20 μL. The calibration curve was generated using polyethylene glycol standards (TSK, Japan).

Monosaccharide composition analysis The monosaccharide composition was determined by GC–MS (Agilent 6890-5975, Agilent, Santa Clara, CA, USA) equipped with FID and DB-5MS (30 m × 0.25 mm × 250 μm, Agilent) according to the method described by Wang et al. (1). Sugar identification was done by comparison with 11 standard monosaccharide including arabinose, rhamnose, ribose, fucose, xylose, mannose, fructose, galactose, glucose, galacturonic acid, and glucuronic acid. The relative content of monosaccharide was calculated by the area normalization method according to the chromatogram.

Fourier-transform infrared spectroscopy analysis The major functional groups were detected by Fourier-transform infrared spectroscopy (FT-IR) spectroscopy (Bruker Tensor 27 instrument, Bruker, Rheinstetten, Germany). The EPS samples were pressed into KBr pellets at a sample: KBr ratio of 1: 100. The FT-IR spectrum was recorded in the region of 4000–400 cm^{-1} (1).

NMR spectroscopy analysis Ten milligram of dried EPS samples were dissolved with 0.5 mL D_2O and lyophilized for thrice to perform deuterium exchange. ^1H NMR and ^{13}C NMR spectra were recorded with a Bruker Avance 600 spectrometer with a 5-mm TXI probe operating at 600 MHz (9).

Scanning electron microscopic analysis Five milligram of lyophilized EPS samples were fixed to the scanning electron microscopic (SEM) stubs with double sided tape, then coated with a layer of gold, ∼10 nm thick. The samples were observed in a scanning electron microscope (S-4800, Hitachi Ltd., Tokyo, Japan) at an accelerating voltage of 3.0 kV.

Atomic force micrograph (AFM) analysis A stock solution (1 mg/mL) was prepared by adding the EPS into distilled H_2O. The aqueous solution was stirred for 1 h at 40°C in a sealed bottle under N_2 stream so that EPS dissolved completely. After cooling to room temperature, the solution was continuously diluted to the final concentration of 0.01 mg/mL. About 5 μL of diluted EPS solution was dropped on the surface of a mica sample carrier, allowed to dry at a room temperature. The atomic force micrograph (AFM) images were obtained using a Dimension Icon instrument (Bruker) in tapping mode.

DPPH radical scavenging activity The DPPH radical scavenging activity was measured by using the method described by Wang et al. (2) minor modifications. Briefly, 1.0 mL of EPS sample solution at gradient concentrations (0.2, 0.5, 1.0, 2.0, 4.0, 7.0, and 10.0 mg/mL) was mixed with 2.0 mL of 0.2 mM freshly prepared DPPH ethanolic solution. Then the mixture was mixed vigorously and incubated at room temperature (RT) in the dark for 1 h. After that, the absorbance of the mixture was measured at 517 nm, and ascorbic acid was used as a positive control. The DPPH radical scavenging ability was calculated via formula.

$$\text{Scavenging activity (\%)} = [1 - (A_1 - A_i)/A_0] \times 100 \tag{1}$$

where A_1 was the absorbance of the sample mixed with DPPH ethanolic solution, A_i was the absorbance of the sample mixed with alcohol, and A_0 was the absorbance of mixture solution without sample.

ABTS radical scavenging activity The ABTS radical scavenging activity was measured by using the method described by Ma et al. (31) minor modifications. Briefly, the $ABTS^{\bullet +}$ was produced by the reaction of 7 mM ABTS in water with 2.49 mM potassium persulfate, then stored in dark at room temperature for 16 h. The $ABTS^{\bullet +}$ solution was then diluted by ethanol to absorbance of 0.70 at 734 nm before use. EPS sample solution (10 μL, 0.2–10.0 mg/mL) was added into 190 μL $ABTS^{\bullet +}$ solution. Then the mixture was incubated for 5 min in dark at RT. The absorbance of the mixture solution was determined at 734 nm, and ascorbic acid was used as a positive control. The calculation formula was same with DPPH radical scavenging activity test.

Hydroxyl radical scavenging activity The hydroxyl radical scavenging activity was measured with the Fenton reaction (2). Briefly, 1 mL of EPS sample solution (0.2–10.0 mg/mL), 1.0 mL of brilliant green (0.435 mM), 2.0 mL of $FeSO_4$ (0.5 mM), and 1.5 mL of H_2O_2 (3.0%, w/v) were added to tubes and mixed to initiate the reaction. The tubes were incubated at 37°C for 30 min. Then, the absorbance was measured at 624 nm, and ascorbic acid was used as a positive control. The hydroxyl radical scavenging activity was calculated via formula.

$$\text{Scavenging activity (\%)} = [(A_0 - A_1)/(A - A_1)] \times 100 \tag{2}$$

where A_0 was the absorbance of mixture solution with sample, A_1 was the absorbance of mixture solution without sample, and A was the absorbance of mixture solution without sample and Fenton reaction system.

Metal chelating activity The metal chelating activity was determined according to the method previously reported by Wang et al. (17) with some modifications. The reaction mixture containing 2.75 mL of water, 1 mL of EPS sample solution (0.2–10.0 mg/mL), 0.2 mL of ferrozine solution (5 mM), and 0.05 mL of $FeCl_2$ solution (4 mM) was shaken vigorously and incubated at RT for 10 min. After that, absorbance of the reaction mixture was measured at 562 nm, and ethylene diamine tetraacetic (EDTA) was used as a positive control. The chelating ability of ferrous ions was calculated via formula.

$$\text{Chelating ability (\%)} = [A_0 - (A_1 - A_i)/A_0] \times 100 \tag{3}$$

where A_0 was the absorbance of mixture solution without sample, A_1 was the absorbance of mixture solution with sample, and A_i was the absorbance of mixture solution without $FeCl_2$.

Oxygen radical absorbance capacity assay The oxygen radical absorbance capacity (ORAC) assay was determined as described by Zheng et al. (32), and glutathione (GSH) was used as a positive control. The concentrations of EPS sample were 40, 80, 120, 160 and 200 μg/mL.

Statistical analysis One-way ANOVA was performed with the Tukey–Kramer post-hoc test to compare the results and all the data were expressed as mean ± standard deviation (SD). Differences at $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Extraction and purification of EPS The crude EPS from the SDM supernatant of *L. plantarum* JLAU103 were prepared through deproteinization and ethanol precipitation. The maximum yield of crude EPS was approximately 75 mg/l after 24 h of incubation in SDM. Then the crude EPS were first separated using a DEAE-Sepharose Fast Flow column. As a result, total four peaks were detected in the gradient elution chromatogram (Fig. 1A). These fractions were considered as acidic polysaccharides because they were all eluted by NaCl (0.2 M or 0.5 M). According to the results of antioxidant activity (Fig. 1A), the third fraction EPS-3 exhibited higher ($P < 0.05$) hydroxyl radical scavenging rate compared to other three fractions. Therefore, the EPS-3 was used for further purification using a Sepharose CL–6B gel column, and only one symmetrical peak was obtained in chromatogram (Fig. 1B), and corresponding fractions, designated as EPS103, were collected, dialyzed and lyophilized for the following analysis.

The results of chemical composition showed that EPS103 contained 90.16% of carbohydrate, 2.09% of moisture, and 1.08% of sulfuric ester. No protein was detected in EPS103, which was in agreement with the result of UV–vis spectrum of EPS103 showing that no absorptions were observed at 260 nm or 280 nm (data not shown). In addition, uronic acid was also not found in EPS103, which was in agreement with the result of monosaccharide composition due to the absence of corresponding peaks for galacturonic acid and glucuronic acid (Fig. 3B).

Molecular weight of EPS The molecular weight and homogeneity of EPS103 were determined using HPSEC. As shown in chromatogram (Fig. 2), a single and symmetrical peak was observed at the retention time of 14.15 min, which confirmed the homogeneity of EPS103. The molecular weight of EPS103 was determined to be 12.4 KDa, which was similar to those of EPS from *L. plantarum* EP56 (44 KDa) (33) and *L. plantarum* KX041 (38.67 KDa) (17), but lower than those of EPS from *L. plantarum* C88 (1.15 × 10^6 Da) (22) and *L. plantarum* JLK0142 (1.34 × 10^5 Da) (9). These differences in molecular weight may be due to the different bacterial sources, culture conditions and hereditary characters (34). Previous studies showed that the bioactivity of EPS seemed to be affected by its molecular weight (35,36). According to Xu et al. (37), the EPS with low molecular weight seem to be more effective for antioxidant activity when compared with the high molecular weight EPS, therefore, in this study, we estimated that the relatively lower molecular weight of EPS103 may be helpful for its antioxidant activity.

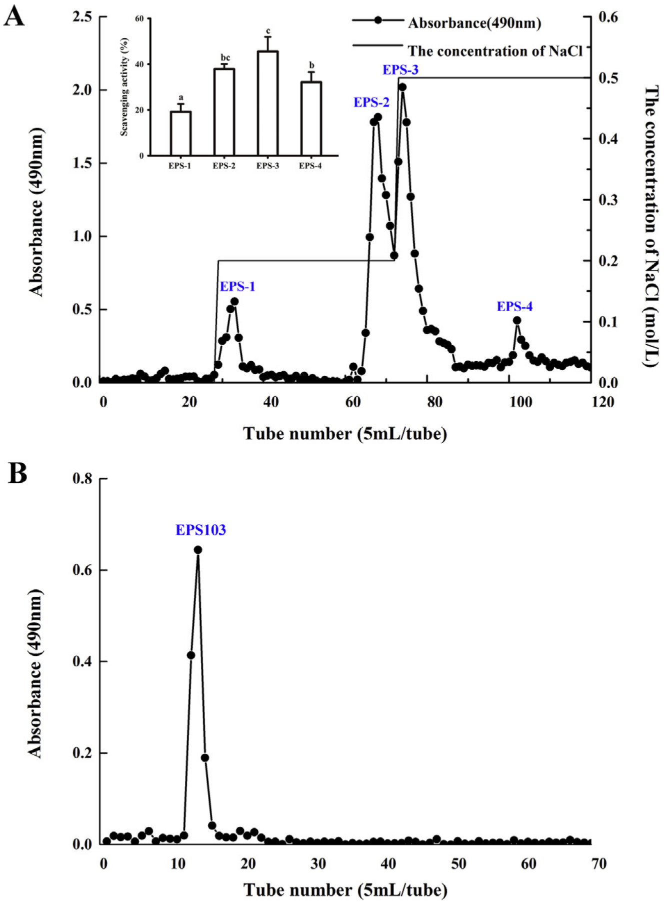

FIG. 1. Chromatograms and hydroxyl radical scavenging activity (insert panel) at various purification steps. (A) Purification of EPS from *L. plantarum* JLAU103 by DEAE-Sepharose Fast Flow column. (B) Purification from the fraction EPS-3 by Sepharose CL-6B column.

Monosaccharide composition of EPS As shown in Fig. 3A, both galacturonic acid and glucuronic acid were not detected when comparing the retention times of the peaks from the standard monosaccharides, indicating that no uronic acids were presented in EPS103, which was also confirmed in chemical composition analysis. GC–MS analysis of monosaccharide composition showed that EPS103 was composed of different monosaccharides including arabinose, rhamnose, fucose, xylose, mannose, fructose, galactose, and glucose in an approximate molar ratio of 4.05:6.04:6.29:5.22:1.47:5.21:2.24:1.83, when comparing the retention times of the peaks from the EPS103 hydrolysate (Fig. 3B) with those of the standard monosaccharides (Fig. 3A), which suggested that EPS103 was a hetero-polysaccharide. As previously reported, most EPS produced by other *L. plantarum* strains were hetero-polysaccharide and were mainly composed of galactose, glucose, mannose, arabinose, fructose and *N*-acetylgalactosamine (22,38,39). Especially, glucose and galactose were the most frequently reported monosaccharides in the EPS from *L. plantarum* (19). This might be associated with the growth media used for the EPS production. In this study, rhamnose and fucose were the predominant monosaccharides in EPS103. Although glucose and galactose were also existed in EPS103, their proportions were not high. Rhamnose was often found in *L. plantarum* EPS, such as the EPS from *L. plantarum* WCFS1, Lp90, and EP56 (15,33,40). However, fucose was rarely found in *L. plantarum* EPS. Since the monosaccharide compositions of EPS have been found to be related to the sources of strains, culture conditions, medium compositions and genetic elements (19), we deduced that the differences in monosaccharide composition of EPS103 from other *L. plantarum* EPS may be due to its specific genetic elements. However, this assumption needs to be proved in our further study.

Infrared spectrum analysis of EPS As shown in Fig. 4, the FT-IR absorption spectrum of EPS103 exhibited a variety of typical absorption peaks of polysaccharides over the range 4000–400 cm^{-1}. A broad, rounded and intense stretching peak at 3425.79 cm^{-1} confirmed the presence of a significant number of hydroxyl groups (O-H), which also suggested that the compound was a polysaccharide (9). Another weak peak at 2922.30 cm^{-1} was due to C-H stretching vibration (17). The strong intensive peak at 1628.18 cm^{-1} was attributed to bound water, and the absorption peak at 1423.06 cm^{-1} was characteristic of carboxyl groups or carboxylates, further indicating that EPS103 was an acidic polysaccharide (41). The absorption peak at 1334.83 cm^{-1} might be assigned to the symmetric stretching vibration of $-COO^-$ groups (42). The absorption at 1115.18 cm^{-1} was possibly due to C-O-C and C-O stretching vibrations and thus confirmed the presence of carbohydrates (1). The absorption at 875.79 cm^{-1} was corresponding to the existence of heteropolysaccharide compounds (43). Overall, based on the FT-IR spectrum, we indicated that the major functional groups including carboxyl and hydroxyl groups which were essential for formation of ropy EPS were present in EPS103.

NMR spectrum analysis of EPS The ^1H and ^{13}C NMR spectra analysis were applied to reveal the complex nature of EPS103. As

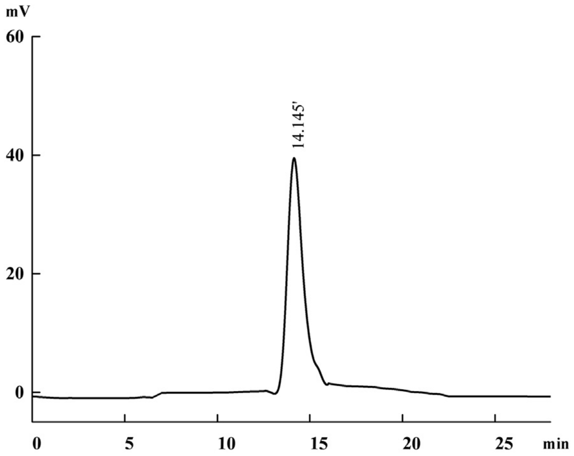

FIG. 2. High performance size exclusion chromatography of purified EPS103 from *L. plantarum* JLAU103.

shown in Fig. 5A, the ^{1}H NMR spectrum of EPS103 can be divided into three regions: the signals between δ 4.4–5.4 ppm corresponded to anomeric region; the ring proton region δ 3.1–4.4 ppm corresponded to the protons linked to C2–C6, and the signals between δ 1.2–2.4 ppm were assigned to the presence of alkyl (44). The signals at downfield regions (δ 4.4–5.4 ppm) corresponded to the anomeric protons of the EPS, and this help to differentiate complex polysaccharide structures. In detail, five anomeric signals were detected at δ 5.10, 5.04, 4.98, 4.81, and 4.51 ppm. The anomeric protons at δ 5.10, 5.04, and 4.98 ppm were the typical, characteristic α-(1→6) chain-extending anomeric signal for EPS (45). The signal at 4.81 ppm was due to the presence of β-type configurations, and the signal at 4.51 ppm indicated the α-D-galactopyranose units (46). In addition, the intensive peak at δ 4.70 ppm was attributed to the solvent hydrogen-deuterium oxide (HDO). The complexity and heterogenous nature of EPS103 was confirmed due to the existence of upfield chemical shifted signals around δ 3.1–4.4 ppm (9). The ^{13}C NMR spectrum (Fig. 5B) of EPS103 included anomeric carbons regions (δ 95–110 ppm) and ring carbons (δ 50–85 ppm) regions. Four chemical shifts were observed at δ 102.44, 101.36, 100.05, and 98.90 ppm. The anomeric carbon signal at 98.90 ppm confirmed that the sugar residues were α-glycosidically linked (1). The shift at δ 76.94 indicated the presence of β-D-glucose and the anomeric carbon signal at δ 102.44 represented the β-D-galactose (44).

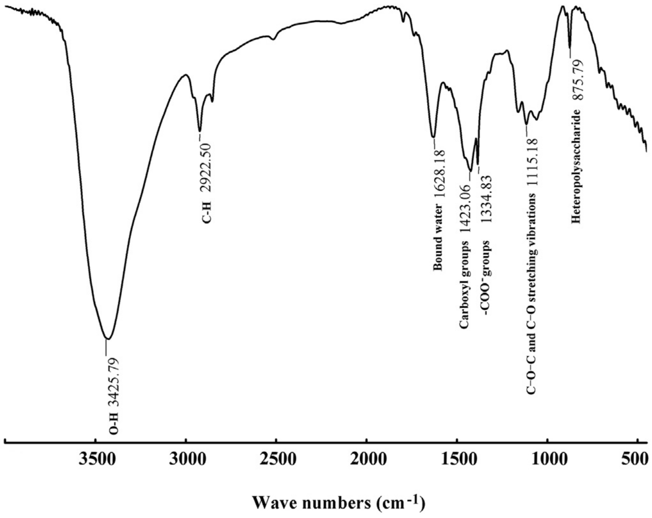

FIG. 4. Fourier-transform infrared spectrum of purified EPS103 from *L. plantarum* JLAU103 over the range 4000–400 cm^{-1}.

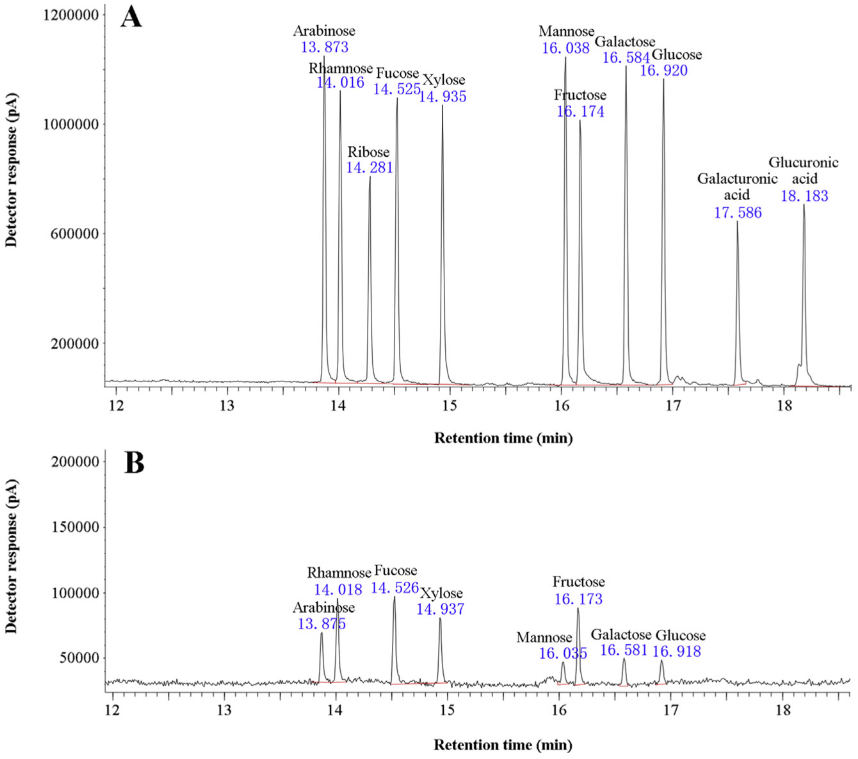

FIG. 3. Gas chromatograms of standard monosaccharides (A) and purified EPS103 from *L. plantarum* JLAU103 (B).

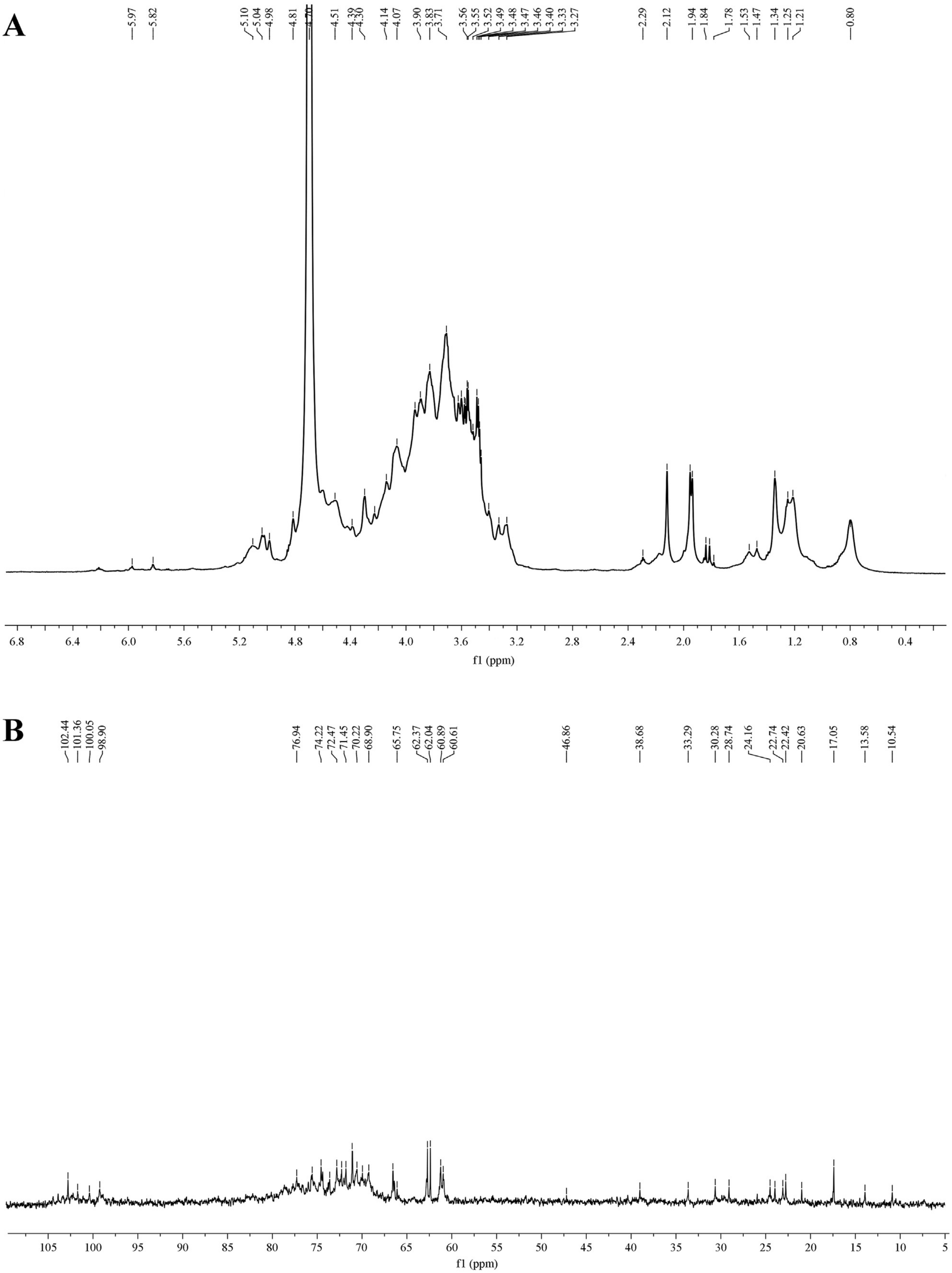

FIG. 5. ^{1}H NMR (A) and ^{13}C NMR spectra (B) of purified EPS103 from *L. plantarum* JLAU103 recorded on Bruker Avance 600 MHz spectrometer in D_2O.

Microcosmic morphology analysis of EPS Observation by SEM of EPS103 (Fig. 6A) revealed a compact structure composed of similar size of cubes. At a higher magnification (20,000 ×), additional details were visible (Fig. 6B). EPS103 exhibited a smooth and glittering cube surface, which was similar to that of EPS produced by *L. plantarum* SKT109 (47). However, observations of other EPS from *L. plantarum* YW11 or *L. plantarum* WLPL04 with the web-like or sheet-like structure (1,48). The EPS103 from *L. plantarum* JLAU103 had the potential to be used in plasticized films manufacturing attribute to its highly compact and smooth surface structure.

Recently, polysaccharides have been extensively studied by using AFM that provides a powerful tool to characterize the morphological topographies of polymers (49). The AFM images of EPS103 are shown in Fig. 6C and D. EPS103 deposited from a 10 μg/mL aqueous solution exhibited many homogeneous rod-shaped lumps with height ranged from 9.6 to 25.0 nm, which was significantly greater than that of a single polysaccharide chain (approximately 0.1−1 nm), suggesting that inter- and/or intra-molecular aggregation might be involved. Similar structural property of EPS was also observed in *L. plantarum* YW11, *L. plantarum* WLPL04, and *L. plantarum* YW32 (1,2,48). This aggregation characteristic of polysaccharides is beneficial to improve its viscous behavior when used in fermented dairy foods.

Antioxidant activity of EPS Hydroxyl radical easily react with neighboring cells or biological molecules and cause serious oxidative damage to cells or biological molecules, leading to diseases such as aging and cancer (17). Recently, some EPS isolated from various lactobacillus were found to possess good hydroxyl radical scavenging activities (36,50,51). As shown in Fig. 7A, both EPS103 and ascorbic acid showed high scavenging abilities on hydroxyl radical. The scavenging percentage was significantly ($P < 0.05$) increased with increasing concentrations of EPS103 and reached the maximum of 80.4% at 10 mg/mL concentration, which was close to the scavenging percentage of ascorbic acid (97.2%) at the same concentration. Similar patterns for hydroxyl radical scavenging were also found in the EPS of *L. plantarum* C88 (85.21%), *L. plantarum* 70810 (68.81%), and *L. plantarum* YW32 (77.5%) (2,22,52). These results suggested that the EPS from *L. plantarum* possessed the potential to be used as natural antioxidants due to their strong scavenging abilities against hydroxyl radical. However, the mechanism of *L. plantarum* EPS in scavenging hydroxyl radical has not been clearly understood. Previous literature reported that EPS could inhibit the formation of hydroxyl group by chelating Fe^{2+} or Cu^{2+} ions which were powerful pro-oxidants and played an important role in the catalytic oxidation reaction (17).

ABTS radical scavenging activity is widely used to determine the antioxidant activity of chemical components (31). The scavenging abilities of ascorbic acid and EPS103 against ABTS radicals are shown in Fig. 7B. The scavenging activity of EPS103 exhibited a concentration-dependent manner and was much lower than that of ascorbic acid at same concentrations. The maximum scavenging percentage of ascorbic acid (99.5%) was obtained at only 2.0 mg/mL concentration. However, EPS103 exhibited more than 50.0% ABTS radical scavenging percentage when the concentration exceeded 7.0 mg/mL, and reached the maximum of 65.5% at the concentration of 10.0 mg/mL. These results indicated that EPS103 had scavenging activity on ABTS radical.

As shown in Fig. 7C, similarly to the trends in the scavenging activities of hydroxyl and ABTS radicals, the DPPH radical scavenging activities of EPS103 and ascorbic acid also exhibited a concentration-dependent manner, with a significantly ($P< 0.05$) increase as the concentration increased from 0.2 to 10.0 mg/mL. The maximum scavenging percentages of EPS103 (60.5%) and ascorbic acid (98.2%) were observed at concentration of 10 mg/mL. The results implied that EPS103 performed high DPPH radical scavenging ability probably by donating electron or hydrogen to DPPH radical and thus terminating the radical chain reaction (53).

Metal chelating activity is one of the antioxidant mechanisms that reduce the concentration of transition metals during lipid

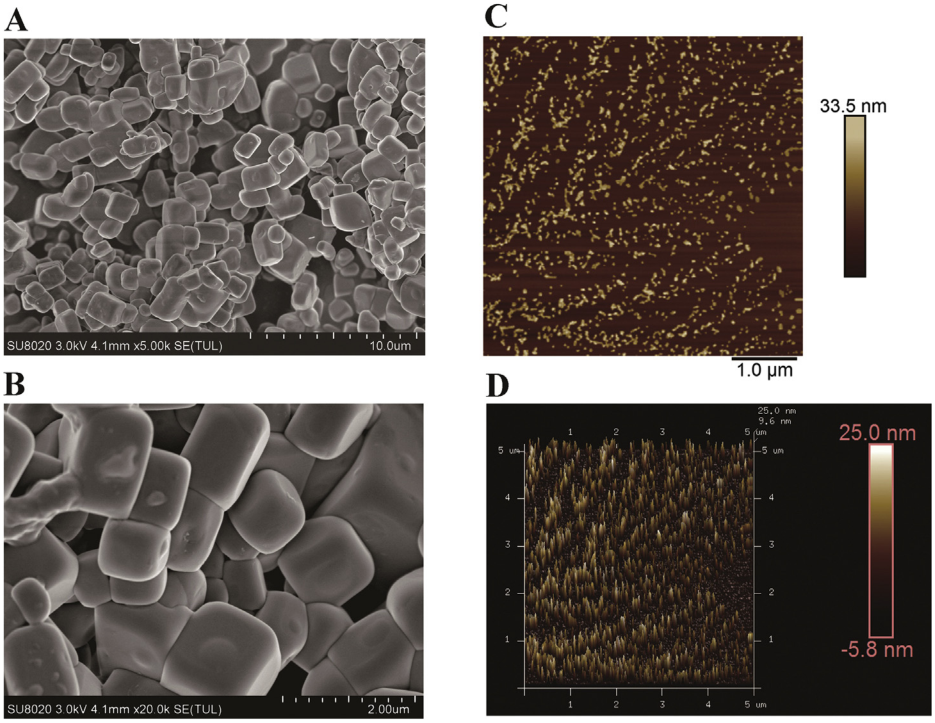

FIG. 6. Scanning electron microscopic images (A: bars: 10 μm; B: bars: 2 μm) and atomic force micrograph images (C: planar, bar: 1 μm; D: cubic) of purified EPS103 from *L. plantarum* JLAU103.

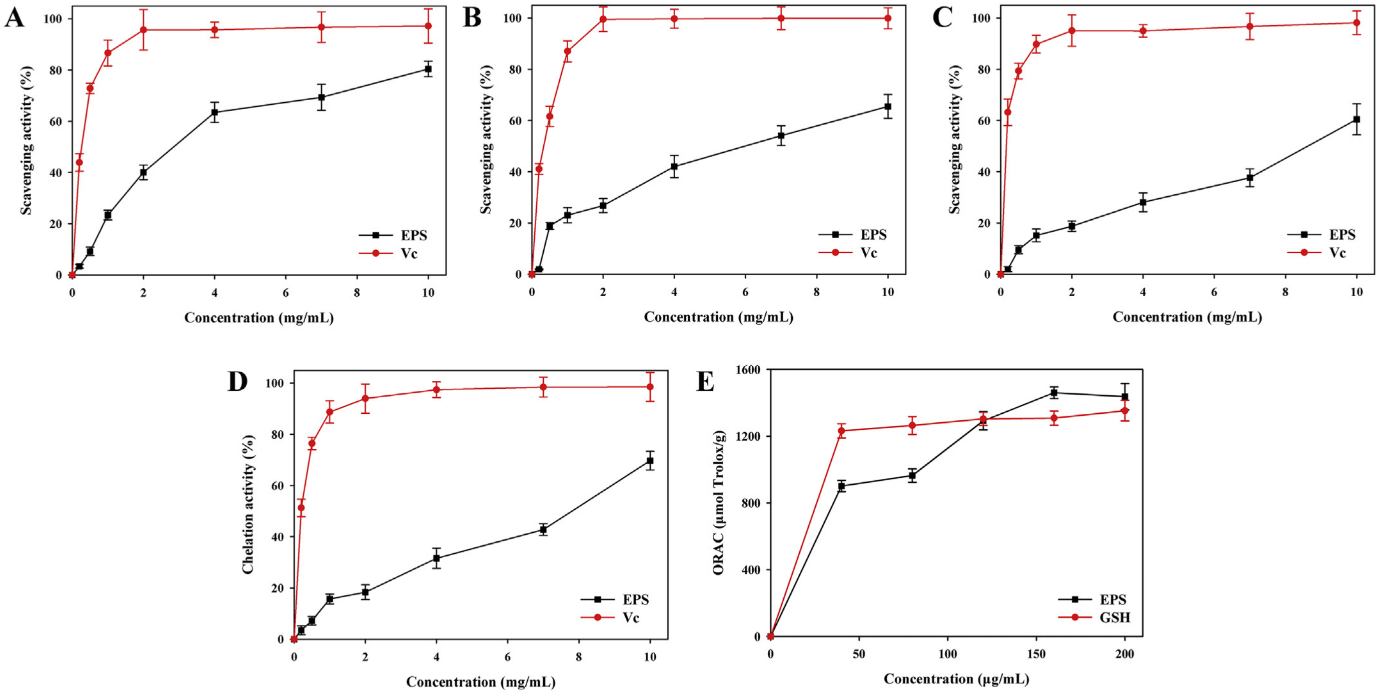

FIG. 7. *In vitro* antioxidant activity of purified EPS103 from *L. plantarum* JLAU103 using ascorbic acid (Vc) and glutathione (GSH) as reference, respectively. (A) Hydroxyl radical scavenging activity, (B) ABTS radical scavenging activity, (C) DPPH radical scavenging activity, (D) ferrous ions chelating ability, and (E) oxygen radical absorbance capacity (ORAC).

peroxidation (10). The results illustrated that ferrous ions could be combined with all tested concentrations of EPS103 and ascorbic acid in a concentration-dependent manner (Fig. 7D). The ferrous ions chelating activities of EPS103 and ascorbic acid both increased to the maximal values of 69.7% and 98.5%, respectively, when their concentrations reached to 10 mg/mL. These results suggested that EPS103 not only act as electrons donor to directly react with free radicals, but also performing antioxidant activity by chelating with transition metal ion catalysts.

ORAC assay is conventionally used to assess the hydrogen atom donating capacity. ORAC values obtained for the EPS103 and GSH are shown in Fig. 7E. EPS103 exhibited the highest ORAC value (1460.50 μmol TE/g) at concentration of 160 μg/mL, which was even higher than those of GSH (1352.87 μmol TE/g, 200 μg/mL). So far we have not found any report on ORAC assay of EPS. However, the ORAC values of defatted peanut meal hydrolysate and defatted walnut meal were 1160 μmol TE/g and 1428.13 μmol TE/g, respectively (32,54). Compared with the data found from other authors, our results suggested that EPS103 might be a good potent antioxidant.

The antioxidant activity of EPS may be affected by various factors including monosaccharide composition, molecular weight, functional groups as well as the extraction and purification methods used (2). As reported earlier, the acidic polysaccharides with lower molecular weight often showed stronger antioxidant activities than the neutral polysaccharides with lower molecular weight (55,56). Furthermore, the antioxidant ability of the polysaccharide was found to be correlated with the protein and uronic acid contents (56,57). In the present study, the results of *in vitro* antioxidant activity suggested that the EPS103 from *L. plantarum* JLAU103 exhibited powerful scavenging effects on hydroxyl, ABTS, and DPPH free radicals, as well as strong chelating power on ferrous ions, and relatively high ORAC value, which demonstrated that this EPS had a great potential to be used as natural antioxidant or functional food additive. According to the results, we deduced that EPS103 performed the antioxidant activity may be attributed to multiple mechanisms including acting as electrons donor to directly react with free radicals, chelating with transition ferrous ion catalysts, and directly absorbing oxygen radicals. Moreover, the presence of a number of hydroxyl groups in EPS103 might provide hydrogen atoms that combined with hydroxyl radicals to form water, and the carbon free radicals could be further oxidized to peroxy radicals, finally decomposed into harmless products (58). However, the antioxidant mechanism of EPS103 has not been clearly understood and future studies should focus on the relationship between the antioxidant mechanism and the structure. In addition, the *in vivo* antioxidant activity of EPS103 should also be further investigated.

Conclusion In this study, an acidic EPS (designated as EPS103) produced by *L. plantarum* JLAU103 was isolated, purified and characterized. The molecular weight of EPS103 was relatively lower of 12.4 KDa EPS103 was hetero-polysaccharide composed of arabinose, rhamnose, fucose, xylose, mannose, fructose, galactose, and glucose. The functional groups of EPS103 were identified by using FT-IR and NMR spectra. SEM and AFM micrographs revealed the smooth cube structure, and homogeneous rod-shaped lumps nature of EPS103. *In vitro* antioxidant activity of EPS103 suggested that this EPS had a great potential to be used as natural antioxidant or functional food additive. Further studies should be performed to define the structure characterization and explore the antioxidant mechanism of EPS103.

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