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Antioxidant and antibacterial activities of exopolysaccharides from *Bifidobacterium bifidum* WBIN03 and *Lactobacillus plantarum* R315

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ABSTRACT

The objective of this study was to investigate the antioxidant and antibacterial activities of exopolysaccharide (EPS) from *Bifidobacterium bifidum* WBIN03 (B-EPS) and *Lactobacillus plantarum* R315 (L-EPS). The 1,1-diphenyl-2-picrylhydrazyl (DPPH)-radical scavenging, hydroxyl radical-scavenging, and superoxide radical-scavenging abilities were measured to evaluate antioxidant activity. Inhibition of erythrocyte hemolysis and lipid peroxidation was also measured. Both B-EPS and L-EPS had strong scavenging ability against DPPH and superoxide radicals at high concentration. The inhibitory effect of B-EPS on erythrocyte hemolysis was stronger than that of L-EPS in a concentration range from 0.30 to 1.00 mg/mL, whereas the hydroxyl scavenging ability of L-EPS (39.15 ± 0.58%) was significantly higher than that of 0.15 mg/mL ascorbic acid (24.33 ± 1.17%) and B-EPS (17.89 ± 3.30%) at 0.10 mg/mL. The inhibition of lipid peroxidation of 0.50 mg/mL B-EPS and L-EPS was 13.48 ± 1.74% and 12.43 ± 0.51%, respectively, values lower than that of ascorbic acid at the same concentration (23.20 ± 1.41%). Furthermore, all these abilities were enhanced in a concentration-dependent manner. Agar diffusion assay showed that both EPS exhibited antibacterial activities against tested pathogens such as *Cronobacter sakazakii*, *Escherichia coli*, *Listeria monocytogenes*, *Staphyloccocus aureus*, *Candida albicans*, *Bacillus cereus*, *Salmonella typhimurium*, and *Shigella sonnei* at 300 μg/mL. In conclusion, both EPS have antimicrobial and antioxidant activities and could have applications in the food industry.

Key words: exopolysaccharide, antioxidative and antibacterial activity, *Lactobacillus plantarum*, *Bifidobacterium bifidum*

INTRODUCTION

Reactive oxygen species such as superoxide, hydroxyl radical, and hydrogen peroxide are highly reactive species, and a major cause of ill effects in humans, including cancer, atherosclerosis, rheumatoid arthritis, and neurodegenerative diseases. These conditions are caused by the damaging effects of reactive oxygen species on biological molecules such as DNA, lipids, and proteins (Ames et al., 1993; Aruoma, 1998). Concern regarding the safety and toxicity of synthetic antioxidants has increased; hence, researchers are challenged to find natural antioxidants that do not adversely affect human health (Valentão et al., 2002; Soubra et al., 2007). In recent years, polysaccharides from yeast, fungi, and medicinal plants have been reported to possess antioxidant activities and could be used as natural antioxidants (Kogan et al., 2008; Yin et al., 2010).

Food poisoning and food spoilage are the most important global issues for the public (Gram et al., 2002), especially in developing countries. Many pathogenic microorganisms, such as *Staphylococcus aureus*, *Bacillus subtilis*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Candida* spp. have been identified as causative agents for foodborne diseases (Sokmen et al., 2004; He et al., 2010). Emergence of multidrug-resistant bacteria has challenged the search for natural antimicrobial substances to prevent food poisoning and spoilage. Recently, many antimicrobial compounds produced by lactic acid bacteria (**LAB**), including bacteriocins, diacetyl, organic acids, carbon dioxide, and some other low-molecular-weight substances, such as reuterin, reutericyclin, and antifungal peptides, have been studied (Nes et al., 2011). However, few studies have focused on antimicrobial and antioxidant activities of exopolysaccharides (**EPS**) produced by LAB (He et al., 2010; Wu et al., 2010).

Exopolysaccharides, long-chain polysaccharides produced by LAB that are secreted into the growth medium during the metabolic process, have attracted recent attention, mainly due to their potential health-promoting functions (Ruas-Madiedo et al., 2002; Badel et al., 2011; Hidalgo-Cantabrana et al., 2012). Interest

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Table 1. Exopolysaccharide (EPS) production by *Bifidobacterium*, *Lactobacillus*, *Lactococcus* and their growth ability (optical density, OD, at 600 nm) in de Man, Rogosa, and Sharpe (MRS) or MRS with L-cysteine medium

Species/strain	Origin	Growth OD at 24 h	EPS production (mg/L)
Bifidobacterium bifidum WBIN03[2]	Human feces	1.82 ± 0.01	241.20 ± 1.55
Lactococcus lactis WLLA11	Human feces	1.54 ± 0.04[c]	200.72 ± 5.43[c]
Lactobacillus acidophilus WLAC12	Human feces	1.55 ± 0.07[c]	210.94 ± 3.02[cd]
Lactobacillus paracasei WLPA13	Human feces	1.59 ± 0.14[c]	243.44 ± 5.56[ef]
Lactobacillus johnsoni WLJO14	Human feces	1.59 ± 0.06[c]	243.85 ± 3.21[ef]
Lactobacillus plantarum R315	Breast milk	1.85 ± 0.02[d]	290.17 ± 19.72[g]
Lactobacillus plantarum WCFS1	Sourdough	1.66 ± 0.03[c]	174.83 ± 10.42[b]
Lactobacillus delbrueckii L001	ATCC 9649	1.74 ± 0.06[cd]	232.55 ± 8.77[de]
Lactobacillus rhamnosus GG L002	ATCC 7469	1.72 ± 0.04[cd]	218.94 ± 8.99[cd]
Lactobacillus fermentum S001	Sourdough	1.52 ± 0.03[c]	214.48 ± 3.39[cd]
Lactobacillus casei V003	Villi	1.60 ± 0.11[c]	255.62 ± 9.21[ef]
Lactobacillus johnsonii T003	Pig feces	1.55 ± 0.031[c]	263.03 ± 7.89[f]
Lactobacillus gasseri PBZF04	Pig feces	1.52 ± 0.04[c]	255.31 ± 6.32[ef]
Lactobacillus vaginalis PBZB08	Pig feces	0.85 ± 0.08[b]	260.09 ± 19.23[f]
Lactobacillus plantarum PFK03	Villi	0.53 ± 0.16[a]	45.49 ± 12.46[a]

[a–g]Data with different letters within a column are significantly different at $P < 0.05$.

[1]Each value in the table is mean ± SD of 3 trials.

[2]The results for *B. bifidum* have been published previously (Li et al., 2014).

has grown in exploiting the EPS-producing LAB for their possible biological activities and important industrial applications. Recently, many new LAB strains have been found to have antibacterial or antioxidant activities (Shen et al., 2011; Li et al., 2012; Serafini et al., 2013). However, these studies were performed without identifying the specific compounds that contributed to these activities. Similarly, only a few studies have focused on the antioxidative and antibacterial effects of EPS produced by LAB (Xu et al., 2011a; Li et al., 2013).

In our previous study, we found that EPS from *Bifidobacterium bifidum* WBIN03 exhibited significant beneficial effects on gut microbiota (Li et al., 2014). We also observed that *Lactobacillus plantarum* R315 produced EPS more efficiently. In the present study, we examined antioxidant and antibacterial activities of EPS from *B. bifidum* WBIN03 and *Lb. plantarum* R315.

MATERIALS AND METHODS

Microorganisms and Culture Conditions

Twenty-three probiotic organisms used in this study were isolated from various sources and identified (Table 1). *Lactobacillus* and *Lactococcus* were cultured in de Man, Rogosa, and Sharpe broth (**MRS**; Beijing Solarbio Science and Technology Co. Ltd., Beijing, China) medium and *Bifidobacterium* was cultured in MRS medium supplemented with L-cysteine at 0.5 g/L (**MRSc**) at 37°C under anaerobic condition. These strains were selected to evaluate their growth ability and EPS yield.

Eight pathogens—*Candida albicans* Z1, *Cronobacter sakazakii* ATCC29544, *Escherichia coli* O157:H7, *Listeria monocytogenes* CMCC54007, *Staphyloccocus aureus* CMCC26003, *Bacillus cereus* ATCC14579, *Salmonella typhimurium* ATCC13311, and *Shigella sonnei* ATCC25931—were used as indicator organisms. These bacteria were cultured in Luria-Bertani medium at 37°C in a shaker incubator overnight except for *Ca. albicans* Z1, which was cultured in yeast extract-peptone-dextrose medium at 30°C for 36 h.

1,1-Diphenyl-2-picrylhydrazyl (**DPPH**) was purchased from Sigma Chemical Co. (St Louis, MO), and all other reagents used were analytical grade and purchased from Shanghai Chemicals and Reagents Co. (Shanghai, China).

Analysis of Bacterial Growth and EPS Production

Cultivation of each strain for EPS production and bacterial growth was performed as batch cultures in 100 mL of MRS or MRSc broth at 37°C in 250-mL Erlenmeyer flasks. Samples (5 mL of each strain) were withdrawn after 24 h of incubation to measuring absorbance at 600 nm. Exopolysaccharide was isolated from samples according to the method described below.

Extraction, Purification, and Detection of EPS

The methods used for extraction, purification, and detection of EPS are based on the method of Chen et al. (2011). Briefly, each strain was separately inoculated (1%) in 500 mL of MRS or MRSc and incubated at 37°C under anaerobic conditions for 24 h without pH control. Cells were removed after centrifugation (4,000 × *g*, 10 min, 24°C), and the supernatant was precipitated with equal volume of ethanol overnight at 4°C fol-

lowed by centrifugation (8,000 × g, 20 min, 4°C). The precipitates were collected and dissolved in distilled water. Proteins were removed with one-quarter of the volume Sevage solution (chloroform:n-butanol; 4:1) followed by centrifugation (8,000 × g, 10 min, 24°C), and finally the collected EPS was dialyzed against distilled water for 1 d at 4°C. The uniformity of the EPS was examined by gel filtration using a column (75 × 1.5 cm) of Bio-Gel P-30 polyacrylamide gel (Bio-Rad, Hercules, CA; exclusion limit: 35,000 Da). The concentration of EPS was determined by measuring the glucose content using the phenol-sulfuric acid assay method (Dubois et al., 1956).

Antioxidant Activity of EPS by Scavenging DPPH Free Radical

The DPPH free radical-scavenging activities of EPS were determined according to the method of Yin et al. (2010). Briefly, 0.2 mM ethanolic DPPH (Sigma Chemical Co.) radical solution was prepared. An aliquot (1.0 mL) of each sample (with appropriate dilution) was added to 1.0 mL of ethanolic DPPH radical solution. Discoloration was measured at 517 nm in triplicate after incubation in the dark for 30 min. Ascorbic acid was used as the positive control. The percentage of scavenged DPPH radical was calculated using the following formula:

$$\text{Scavenging ability (\%)} = [1 - (A_{sample} - A_{blank})/A_{control}] \times 100,$$

where A_{sample} is the absorbance of the sample, A_{blank} is the absorbance of the blank, and $A_{control}$ is the absorbance of the control. Ethanol (1.0 mL) plus sample solution with different dilutions (1.0 mL) were used as blanks and DPPH radical-ethanol solution (1.0 mL) plus ethanol (1.0 mL) as a negative control.

Antioxidant Activity of EPS by Scavenging Hydroxyl Radical

The test was carried out according to Yin et al. (2010), with a slight modification. Briefly, 2.0 mL of PBS (20 mM, pH 7.4), 1.0 mL of 1,10-phenanthroline solution (2.5 mM), 1.0 mL of $FeSO_4$ solution (2.5 mM), and 1 mL of H_2O_2 (20 mM) were added successively in a tube and mixed thoroughly. Then, 1 mL of EPS aliquot with various concentrations was added in the mixture and incubated at 37°C for 1 h. The absorbance was measured at 536 nm immediately and hydroxyl radical scavenging activity was expressed as

$$\text{Scavenging activity (\%)} = [(A_S - A_C)/(A_O - A_C)] \times 100,$$

where A_S is the absorbance in the presence of the sample, A_C is the absorbance of the control in the absence of the sample, and A_O is the absorbance without both sample and H_2O_2.

Antioxidant Activity of EPS by Scavenging Superoxide Radical

This assay was performed according to Zhang et al. (2011), with minor modifications. An aliquot of 2.0 mL of Tris-HCl buffer (pH 8.0, 150 mM) was mixed with 1.0 mL of 1,2,3-phentriol (1.50 mM, dissolved with 10 mM HCl) and 0.5 mL of sample solutions with different concentrations. After thorough mixing, the mixture was incubated at room temperature for 30 min. The absorbance of the mixture was measured at 325 nm and superoxide radicals scavenging generated by 1,2,3-phentriol autoxidation was calculated as follows:

$$\text{Scavenging effect (\%)} = [1 - (A_{11} - A_{10})/(A_{01} - A_{00})] \times 100,$$

where A_{00} is the absorbance of the sample in the absence of EPS and 1,2,3-phentriol, A_{01} contained 1,2,3-phentriol but no EPS, A_{10} contained EPS but no 1,2,3-phentriol, and A_{11} contained EPS and 1,2,3-phentriol.

Inhibition of Lipid Peroxidation

This assay was carried out according to the method of Yin et al. (2010). The yolk suspension was prepared according to Xu et al. (2011b). One milliliter of yolk suspension was mixed with 1.0 mL of different concentrations of sample, 0.4 mL of 25 mM $FeSO_4$, and 2.6 mL of PBS followed by incubation under shaking at 37°C for 15 min. The reaction was stopped by adding 1.0 mL of 20% TCA and heating at 100°C for 15 min with 2 mL of 0.8% thiobarbituric acid. After centrifugation (3,000 × g, 10 min, 24°C) to remove precipitated proteins, the absorbance was measured at 532 nm. The inhibition capability of lipid peroxidation was calculated as follows:

$$\text{Inhibition effect (\%)} = (A_C - A_S)/A_C \times 100,$$

where A_C is the absorbance of the control and A_S is the absorbance in the presence of samples.

Inhibition of Erythrocyte Hemolysis

The protocol for carrying out animal experiments was approved by Nanchang University Animal Ethics Com-

mittee and all the ethical requirements to conduct the experiment were met. All procedures were conducted in compliance with protocols provided and approved by the Animal Care Review Committee (approval number 0064257) of Nanchang University (Jiangxi, China).

Blood samples were collected from Kunming mice (BW of 25.0 ± 2.0 g) by plucking the eyeball (retro-orbital blood collection), and then, a 1.25% erythrocyte suspension was obtained according to Saengkhae et al. (2007).

The inhibition of mice erythrocyte hemolysis by EPS was evaluated according to the procedures of Xu et al. (2011b) with a slight modification. Briefly, different concentrations of samples were incubated at 37°C for 30 min with 2.0 mL of erythrocyte suspension and 1.0 mL of H_2O_2 (2.5 m*M*). Then, the mixture was incubated in a 0°C ice water bath immediately to terminate the reaction and centrifuged at 3,000 × *g* for 10 min at 24°C. The absorbance was measured at 540 nm. In the control testing, H_2O_2 and the sample were substituted with Tris buffer. In the H_2O_2 control, the sample was substituted with Tris buffer.

The extent of hemolysis of the erythrocyte was calculated as follows:

$$\text{Extent of hemolysis (\%)} = A_{\text{sample}} / A_{H_2O_2} \times 100,$$

where A_{sample} is the absorbance of the sample and $A_{H_2O_2}$ is the absorbance of the H_2O_2 control.

The inhibition rate of the protein was calculated by the following equation:

$$\text{Inhibition rate (\%)} = \left(A_{H_2O_2} - A_{\text{sample}}\right) / \left(A_{H_2O_2} - A_{\text{normal}}\right) \times 100,$$

where A_{normal} is the absorbance of the control and other terms are as defined previously.

Antimicrobial Activities of EPS

The antibacterial activity of EPS was investigated by agar diffusion assay as described by Chen et al. (2014) with a slight modification. Overnight incubation cultures of the indicator microorganisms were diluted to 10^6 to 10^7 cfu/mL and then spread on 10 mL of Luria-Bertani or yeast extract-peptone-dextrose agar in a Petri dish. Then, 200 μL of EPS (300 μg/mL) was added into an Oxford cup (a stainless cylinder of o.d. 7.8 ± 0.1 mm, i.d. 6.0 ± 0.1 mm, and height 10.0 ± 0.1 mm), which was placed on the surface of the agar. The incubation temperature was as described in the section "Microorganisms and Culture Conditions." The size of the clear zone around the cup (including that of the Oxford cup, 7.8 mm) was measured. The experiment was performed in triplicate, and ampicillin (100 μg/mL) was used as a positive control.

Statistical Analysis

Data were statistically analyzed using the one-way ANOVA procedure of SPSS (version 13.0; IBM Corp., Chicago, IL) and expressed as mean ± SD. All these experiments were performed in triplicate and within each replication; analyses were carried out in duplicate. The differences among means were tested by the Student-Newman-Keuls test. Data were considered statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

EPS Production and Bacterial Growth Ability

The growth properties of bacteria and EPS yields of each strain were investigated and are shown in Table 1. *Bifidobacterium bifidum* WBIN03 produced the greatest amount of EPS (241.20 ± 1.55 mg/L) compared with other *Bifidobacterium* in MRSc medium, and also possessed the best growth ability (OD_{600} was 1.82 ± 0.01; Li et al., 2014). All the *Lactobacillus* strains and one *Lactococcus* strain used in this study produced EPS, and the amount of the EPS ranged from 45.49 ± 12.46 to 290.17 ± 19.27 mg/L. In addition, *Lb. plantarum* R315 produced much higher levels of EPS (290.17 ± 19.27 mg/L) compared with other *Lactobacillus* strains. It also grew better in MRS (optical density at 600 nm was 1.85 ± 0.02) than other strains ($P < 0.01$). Based on these results, *B. bifidum* WBIN03 and *Lb. plantarum* R315 were selected for EPS extraction and further studies.

Antioxidant Activity by Scavenging of DPPH Radicals

The DPPH radical has been used as a tool to investigate the antioxidative activity of antioxidants (Zhang et al., 2013). In this study, the effect of EPS from *B. bifidum* WBIN03 (B-EPS) and EPS from *Lb. plantarum* R315 (L-EPS) on DPPH scavenging ability is shown in Figure 1. The scavenging activity of 0.30 mg/mL B-EPS (60.08 ± 3.01%) was similar to that of 0.25 mg/mL ascorbic acid (62.78 ± 2.19%) and higher than that of L-EPS (56.48 ± 3.10%) at the same concentration. In addition, scavenging activity increased with EPS concentration, and 0.50 mg/mL ascorbic acid (96.16 ± 0.74%) had activity similar to that 0.70 mg/mL B-EPS (94.40 ± 2.15%) and 1.00 mg/mL L-EPS (94.49 ± 0.31%), respectively. These results indicate the strong antioxidative effect of these 2 EPS on DPPH radical scavenging.

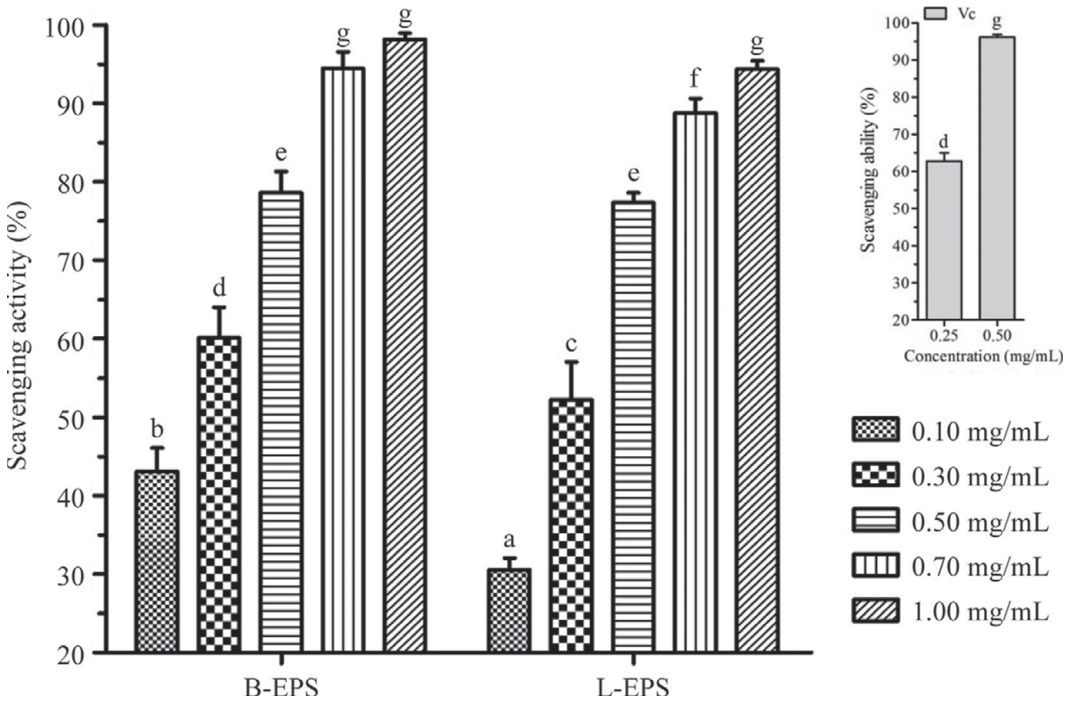

Figure 1. Scavenging effect of B-EPS and L-EPS on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals compared with that of ascorbic acid. Data with different letters are significantly different at $P < 0.05$. B-EPS = EPS from *Bifidobacterium bifidum* WBIN03; L-EPS = EPS from *Lactobacillus plantarum* R315; Vc = ascorbic acid.

Antioxidant Activity by Scavenging of Hydroxyl Radicals

Hydroxyl radical is a powerful oxidant that can react with almost all biological molecules (proteins, lipids and carbohydrates; Santanam et al., 1998). As shown in Figure 2, both B-EPS and L-EPS exhibited concentration-dependent scavenging activities against hydroxyl radicals, and scavenging activity increased with concentration in the range from 0.01 to 1.50 mg/mL. The scavenging ability of 0.10 mg/mL L-EPS (39.15 ± 0.58%) was similar to that of 0.25 mg/mL ascorbic acid (36.48 ± 2.44%) and was significantly higher than that of 0.15 mg/mL ascorbic acid (24.33 ± 1.17%) and B-EPS at all concentrations. In contrast, the scavenging ability of 1.50 mg/mL B-EPS (31.91 ± 1.62%) was only half that of L-EPS (63.16 ± 3.29%) at the same concentration, and was even lower than that of 0.25 mg/mL ascorbic acid (36.48 ± 2.44%). These results suggested that L-EPS had better hydroxyl radical scavenging activity than the reference ascorbic acid.

Antioxidant Activity by Scavenging of Superoxide Radicals

As free radical to cellular components, the superoxide radical is a precursor of more reactive oxidative species; for example, singlet oxygen and hydroxyl radical, and it can also initiate lipid peroxidation (Marklund and Marklund, 1974; Yin et al., 2010). The scavenging activity of B-EPS and L-EPS against superoxide radicals generated by 1,2,3-phetriol autoxidation is shown as Figure 3. The scavenging activities of 0.10 mg/mL B-EPS (20.30 ± 1.91%) and 0.10 mg/mL L-EPS (25.25 ± 2.63%) were similar to that of 0.15 mg/mL ascorbic acid (27.35 ± 5.40%). However, at 1.00 mg/mL, the scavenging activities of B-EPS (59.75 ± 8.12%) and L-EPS (65.45 ± 8.48%) were both slightly higher ($P > 0.05$) than that of ascorbic acid at 0.25 mg/mL (57.38 ± 4.86%). Thus, the superoxide scavenging abilities of B-EPS and L-EPS were similar.

Inhibition of Lipid Peroxidation

Lipid peroxidation is an oxidative deterioration process of PUFA induced by radicals (Su et al., 2009), and it is likely involved in numerous pathological events, including inflammation, metabolic disorder, and cellular aging (Wiseman and Halliwell, 1996). The inhibition of lipid peroxidation by B-EPS and L-EPS is shown in Figure 4. Both EPS exhibited dose-dependent inhibitory activities. At 0.50 mg/mL, the inhibitory activities of B-EPS (13.48 ± 1.74%) and L-EPS (12.43 ± 0.51%) were similar but lower than that of ascorbic acid (23.20 ± 1.41%) at the same concentration; however, little difference was observed for a concentration range of

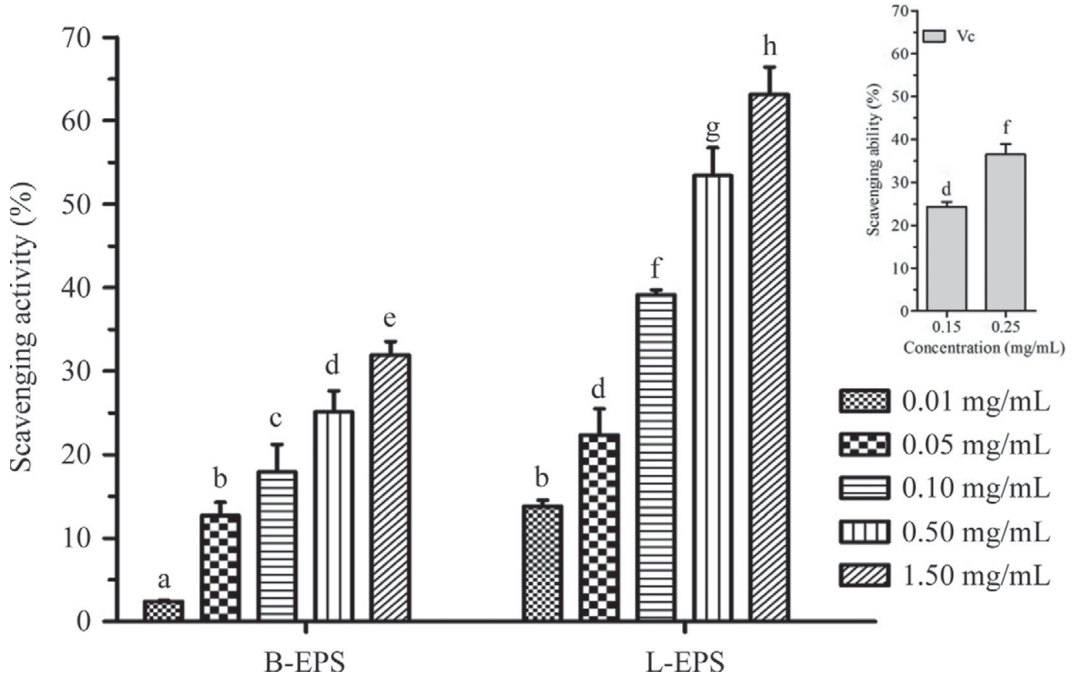

Figure 2. Scavenging effect of B-EPS and L-EPS on hydroxyl radicals compared with that of ascorbic acid. Data with different letters are significantly different at $P < 0.05$. B-EPS = EPS from *Bifidobacterium bifidum* WBIN03; L-EPS = EPS from *Lactobacillus plantarum* R315; Vc = ascorbic acid.

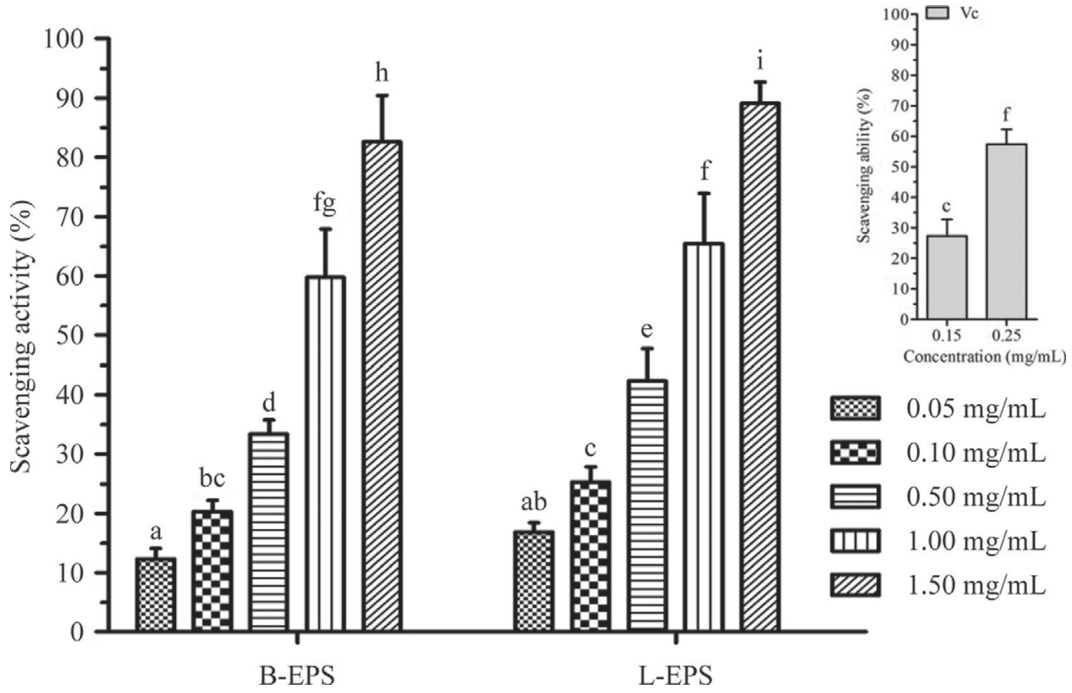

Figure 3. Scavenging effect of B-EPS and L-EPS on superoxide radicals generated by 1,2,3-phentriol autoxidation compared with that of ascorbic acid. Bars with different letters are significantly different at $P < 0.05$; bars with common letters are not significantly different at $P > 0.05$. B-EPS = EPS from *Bifidobacterium bifidum* WBIN03; L-EPS = EPS from *Lactobacillus plantarum* R315; Vc = ascorbic acid.

1.00 to 1.50 mg/mL, which indicated that they had similar inhibitory effect on lipid peroxidation. In the present work, Fe^{2+} induced the production of lipid radicals, and malondialdehyde (MDA) was quantified for determining the extent of lipid peroxidation. Several previous studies indicated that EPS from *Lactobacillus* and *Bifidobacterium* show strong inhibitory effects on lipid peroxidation (Shen et al., 2011; Xu et al., 2011a,b; Li et al., 2012; Guo et al., 2013); however, no details on the mechanism of action have been described.

Inhibition of Mice Erythrocyte Hemolysis

The oxidative hemolysis of erythrocytes from mice was used as the model to investigate the damage of biological membranes induced by free radicals and the protective effect of EPS. In the present study, we investigated the extent of hemolysis and inhibition rate of B-EPS and L-EPS on mice erythrocytes. As shown in Figure 5, the hemolysis rate for B-EPS, L-EPS, and ascorbic acid were >90% at low concentrations (0.10–0.30 mg/mL) but decreased significantly ($P < 0.5$) at concentrations from 0.50 to 1.00 mg/mL. The B-EPS had a stronger inhibitory effect on extent of hemolysis than did L-EPS or ascorbic acid (Figure 5A). Although the inhibition rate of ascorbic acid was 49.93 ± 4.30% at 0.50 mg/mL, B-EPS (56.28 ± 3.30% at 0.70 mg/mL) and L-EPS (42.73 ± 3.15% at 1.00 mg/mL) had similar inhibitory activities at higher concentration (Figure 5B). Several studies have demonstrated that some antioxidants from different materials; for example, natural products, cocoa, *Nelumbo nucifera* Gaertn., *Mangifera indica* L., exopolysaccharide from *Bifidobacterium*, show strong inhibitory effects on erythrocyte hemolysis induced by free radicals in vitro (Ng et al., 2000; Zhu et al., 2002; Saengkhae et al., 2007; Ajila and Prasada Rao, 2008; Xu et al., 2011b). Our results also suggested that EPS from *Lb. plantarum* and *B. bifidum* could protect normal mice erythrocytes against damage in vitro in a dose-dependent manner.

Antimicrobial Activity

Antibacterial activity of B-EPS and L-EPS in samples is shown in Figure 6. Both B-EPS and L-EPS had equal antimicrobial activities against *Listeria monocytogenes* CMCC54007, *Staph. aureus* CGMCC26003, *Bacillus cereus* ATCC14579, and *S. typhimurium* ATCC13311, and the inhibition zones were lower than those with 50 μg/mL ampicillin. For *Cronobacter sakazakii* ATCC29544 and *Shigella sonnei* ATCC25931, the inhibition zones of L-EPS were 17.83 ± 1.04 and 10.67 ± 0.29 mm, respectively, which were significantly greater than those for B-EPS. In contrast, the inhibition zone

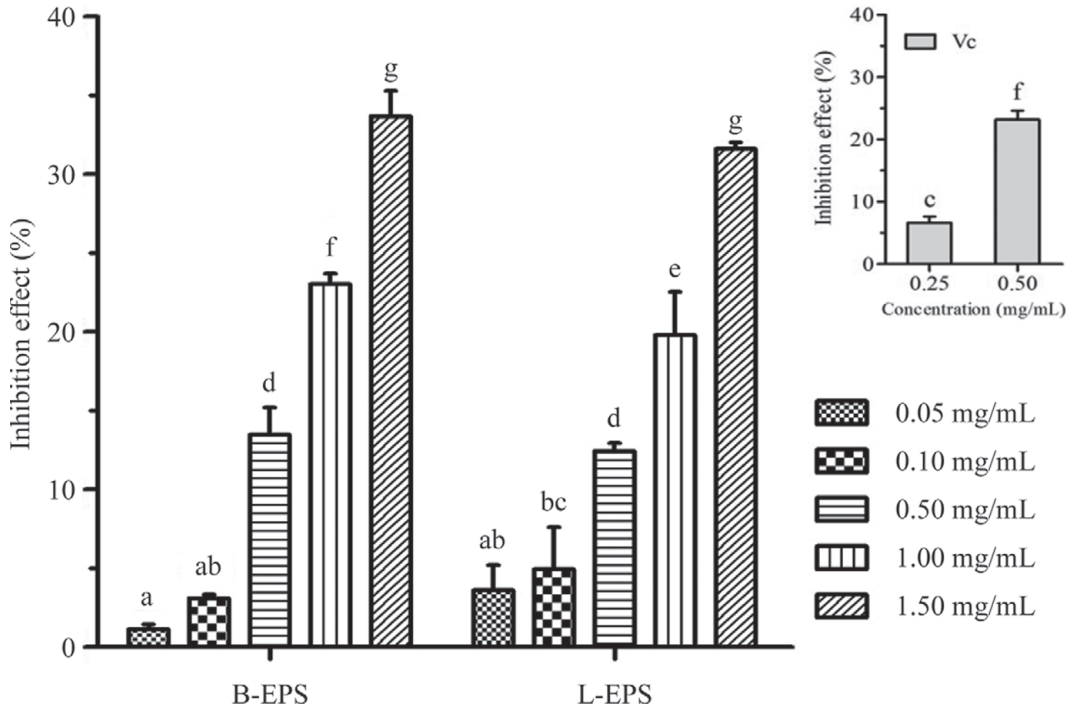

Figure 4. Inhibition of lipid peroxidation by B-EPS and L-EPS compared with that of ascorbic acid. Bars with different letters are significantly different at $P < 0.05$; bars with common letters are not significantly different at $P > 0.05$. B-EPS = EPS from *Bifidobacterium bifidum* WBIN03; L-EPS = EPS from *Lactobacillus plantarum* R315; Vc = ascorbic acid.

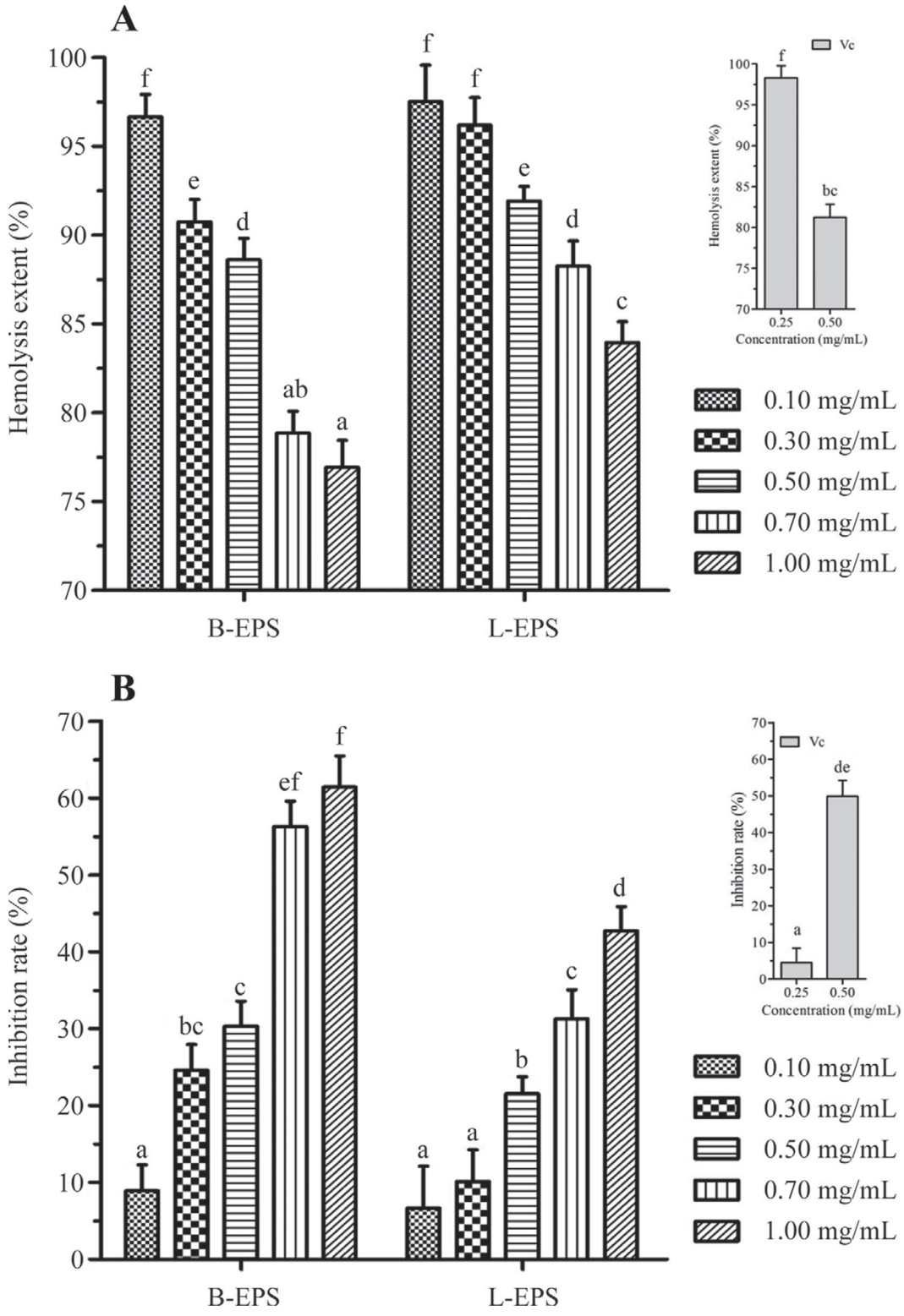

Figure 5. Effects of B-EPS and L-EPS on hemolysis of rat erythrocytes induced by H_2O_2 compared with that of ascorbic acid: (A) extent of hemolysis; (B) inhibition rate. Bars with different letters are significantly different at $P < 0.05$; bars with common letters are not significantly different at $P > 0.05$. B-EPS = EPS from *Bifidobacterium bifidum* WBIN03; L-EPS = EPS from *Lactobacillus plantarum* R315; Vc = ascorbic acid.

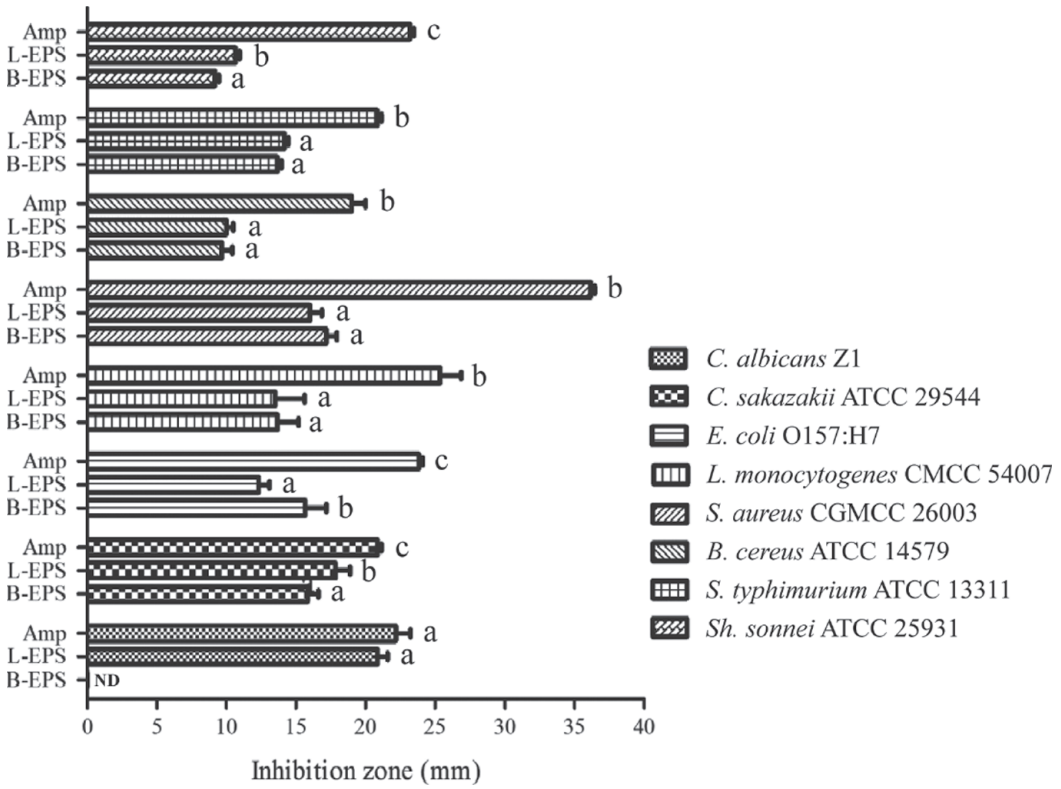

Figure 6. Antibacterial activities of B-EPS (300 μg/mL) and L-EPS (300 μg/mL) compared with that of ampicillin (100 μg/mL). Data with different letters are significantly different at $P < 0.05$; ND = not detectable. B-EPS = EPS from *Bifidobacterium bifidum* WBIN03; L-EPS = EPS from *Lactobacillus plantarum* R315; Amp = ampicillin. Activity was monitored against *Candida albicans*, *Cronobacter sakazakii*, *Escherichia coli*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhimurium*, and *Shigella sonnei*.

of B-EPS for *E. coli* O157:H7 (15.67 ± 1.53 mm) was significantly greater than that of L-EPS (12.00 ± 1.32 mm). Interestingly, L-EPS inhibited the growth of *Ca. albicans* Z1 (20.83 ± 0.76 mm) similarly to ampicillin; however, B-EPS had no effect on *Ca. albicans* Z1. A few studies showed that EPS from microorganisms had strong antimicrobial activity against several pathogens in vitro and have proposed several possible antibacterial mechanisms of EPS, such as impairing cell division, disrupting the cell wall and cytoplasmic membrane, and decomposing DNA (He et al., 2010; Wu et al., 2010). Although those studies did not describe the exact antagonistic mechanism of polysaccharide, protocols from these studies might lead to new areas in which to explore the antibacterial activity of polysaccharides.

CONCLUSIONS

In this study, all probiotic organisms studied could produce EPS under laboratory conditions, and *B. bifidum* WBIN03 and *Lb. plantarum* R315 were able to produce EPS more effectively and exhibited better growth ability in the medium compared with the other strains. The results showed that both B-EPS and L-EPS had strong antibacterial ability against the tested pathogens. Similarly, they showed strong scavenging activity against DPPH, hydroxyl, and superoxide radicals, and inhibitory effects on lipid peroxidation and erythrocyte hemolysis. Although both EPS showed novel biological activities, further studies are needed to evaluate their structure and the components responsible for antimicrobial activity and mode of actions of EPS.

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