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Two Novel Exopolysaccharides from *Bacillus amyloliquefaciens* C-1: Antioxidation and Effect on Oxidative Stress

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Abstract Two odorless, water-soluble exopolysaccharide (EPS) fractions, EPS-1 and EPS-2, were isolated from a newly isolated *Bacillus amyloliquefaciens* strain C-1 and purified by ion exchange and gel chromatography. The purified EPS-1 contained glucose/mannose/galactose/arabinose in a relative proportion of 15:4:2:1, and possessed a molecular weight of 79.6 kDa, while EPS-2 contained only glucose and mannose in a 3:1 ratio, with the molecular weights of 19.8 kDa. The antioxidant activity results showed that EPS-1 exhibited strong reducing power, superoxide radicals ($O^{2-}\cdot$), and hydroxyl free radicals (OH·) scavenging activities. For the H_2O_2-induced injury in HepG2 cells, EPS-1 significantly decreased the formation of reactive oxygen species, intracellular malondialdehyde levels, and restored intracellular superoxide dismutase activity. For EPS-2, there had no detectable antioxidant activities. And all these results collectively showed that as a natural antioxidant, only EPS-1 produced by C-1 had considerable potential to be used as medical compounds or functional additives.

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Introduction

Oxidative stress, induced by oxygen radicals, is considered as a primary factor in various degenerative diseases, including cancer, atherosclerosis, hyperlipidemia, and diabetes [25]. Reactive oxygen species (ROS) and oxygen-derived free radicals, generated by normal metabolic processes or from exogenous factors and agents, may contribute to a variety of pathological effects, including DNA damage, carcinogenesis, and cellular degeneration [21]. Most organisms possess antioxidant defense and repair systems but they are insufficient to prevent DNA or proteins damage. Live cells protect themselves from oxidative damage through several defense mechanisms such as the enzymatic conversion of ROS into less toxic substances or through the antioxidant process [7]. Many synthetic compounds, such as butyl hydroxyl anisd (BHA), butylated hydroxytoluene (BHT), and tertiary butylhydroquinone (TBHQ), are commonly used as antioxidant in processed foods. However, synthetic antioxidants are being restricted due to their side effects such as carcinogenicity [12]. With these safety concerns, the increasing interest has heightened in finding naturally occurring antioxidants to be used in foods or medicinal materials, which had the capacity to improve food quality, terminate free radical chain reactions in biological systems, and provide additional health benefits to consumers [2].

As an important class of bio-active natural polymers, polysaccharides have been considered to be promising antioxidants and candidates of effective, nontoxic medicines and food additives in vitro and in vivo [32]. Exopolysaccharide (EPSs) is one type of metabolite in many microorganisms. They are usually biocompatible, edible, and nontoxic to humans and the environment [22]. Recently, there has been an increased interest in exploiting

the EPSs for their biological activities including antitumor, immunostimulatory, cholesterol-lowering activity, and antioxidant activities [7, 31]. For example, cell-bond exopolysaccharide (cb-EPS) from *Lactobacillus acidophilus* can inhibit the proliferation of HT-29 colon cancer cells, and it possesses great pro-apoptotic activity in oncotherapy and adjuvant therapy [13]. In this current study, we found that fermentation solution produced by *Bacillus amyloliquefaciens* strain C-1 which was isolated from ready-to-eat sliced apple samples had significant antioxidant activities. To elucidate the mechanism of the active component contributed to the antioxidation, two novel water-soluble EPSs produced by C-1 were purified and characterized, and their antioxidant activities were investigated using various assays.

Materials and Methods

Strains and Cells

Bacillus amyloliquefaciens strain C-1 (16S rRNA accession no. JX028840 in GenBank) was isolated from ready-to-eat sliced apple samples by the Food Microbiology Lab of the Nutrition and Food Safety Engineering Research Center of Shaanxi Province, Xi'an, China (the China Center for Type Culture Collection, CCTCCM2012177). HepG2 cells, a human hepatoblastoma cell line, were cultured in high glucose Dulbecco's Modified Eagle's Medium supplemented with 100 U/ml penicillin, 100 U/ml streptomycin, and 10 % fetal bovine serum (FBS), and kept at 37 °C in a humidified atmosphere of 5 % CO_2. All cells were plated in cell culture flasks at least 24 h before treatment.

Morphological Properties and 16S rRNA Gene Sequence Analysis of Strain C-1

Strain C-1 was cultured in LB (Luria–Bertani) medium with 1 % glucose (pH 7.5). The cell morphological feature was observed by scanning electron microscopy (Hitachi, TM-1000) after growth for 60 h.

The 16S rRNA sequence was used for the identification of C-1. Primers used were: 16S-F: 5′AGAGTTTGAT CCTGGCTCAG3′, 16S-R: 5′GGTACCT TGTTACG ACTT3′ [11]. The PCR fragments were analyzed by gel electrophoresis, then purified and sequenced by Sangon Biotech (Shanghai, CN) Co, Ltd. The 16S rRNA sequence was used for the identification of C-1. Multiple alignments with sequences of most close similarity were analyzed using CLUSTAL W, and the phylogenetic tree was constructed by using the neighbor-joining method [9].

EPS Isolation and Purification

A 2 % (v/v) inoculum of *B. amyloliquefaciens* C-1 culture was sub-cultured and grown for 72 h at 30 °C. The fermented culture supernatant was concentrated, and crude EPS was precipitated by addition of 2 volumes of pre-cooled 95 % ethanol at 4 °C [30]. Crude EPS was collected and the pellet was dissolved in deionized water, and deproteinized with Sevag reagent and dialyzed. The dialyzed solution was lyophilized for further EPSs purification. The amount of crude EPSs was estimated calorimetrically by phenol–sulfuric acid method [8].

The freeze-dried sample was fractionated with an anion-exchange chromatography on the DEAE-Cellulose column (2.6 × 30 cm) (Whatman, USA), eluted with a step gradient of NaCl solution (0–1.0 M). Based on the chromatogram detected by the phenol sulfuric acid method, the EPS was resolved into two major peaks and fractions containing polysaccharides were pooled, dialyzed, and lyophilized. Further purification of EPS was performed by gel filtration using a sepharose CL-6B column (2.6 × 100 cm) (Amersham Pharmacia Biotech, Sweden) eluted with 0.9 % (w/v) NaCl. The major polysaccharide fraction was pooled, dialyzed with water, and freeze-dried.

Molecular Mass Determination of EPSs

The average molecular weight of each purified EPS was measured by gel permeation chromatography. Standard dextrans (500, 110, 70, 40, and 10 kDa, Pharmacia, USA) were passed through a sepharose CL-6B column (1.6 × 100 cm) and eluted with 0.9 % (w/v) NaCl at a flow rate of 0.67 ml per min. The elution volumes were plotted against the logarithms of their respective molecular weights. Purified EPSs were dissolved in 0.15 M NaCl and applied to the same column equilibrated.

Monosaccharide Composition of EPS

Five milligram of EPSs was hydrolyzed with 2 ml of 2 mol/l trifluoroacetic acid (TFA) at 120 °C for 2 h. The hydrolyzate was co-concentrated repeatedly with methanol to dryness, reduced with $NaBH_4$ for 30 min at 20 °C, and acetylated with acetic anhydride and pyridine at 100 °C for 20 min. The standard sugars were prepared in the same way. The alditol acetates of EPSs were analyzed on Agilent Technologies 7890A GC equipped with flame ionization detector (FID) and a HP-5 fused silica capillary column (30 × 0.32 × 0.25 mm). The nitrogen gas was used as the carrier gas at a flow rate of 1 ml/min. The column temperature was kept at 120 °C for 2 min and then increased to 250 °C for 3 min at a rate of 8 °C per min [23]. These assays were repeated in triplicates.

Assay of Antioxidant Activity In Vitro

Determination of Reducing Power

The reducing power of EPS-1 and EPS-2 was determined by the modified method from [14]. The reaction mixture, which included 1 ml EPS at different concentrations (0.15, 0.30, 0.6, 1.25, 2.5, and 5 mg/ml), 2.5 ml of 0.2 M sodium phosphate buffer (pH 6.6), and 2.5 ml of $K_3Fe(CN)_6$ (1 %, v/v), was incubated at 50 °C for 20 min before the addition of 2.5 ml of 10 % trichloroacetic acid (TCA); A 2.5 ml volume of the supernatant was mixed with 0.5 ml of fresh $FeCl_3$ and 2.5 ml of sterile H_2O. After 5-min incubation, the absorbance at 700 nm was measured. Vitamin C (Vc) was used as a positive control. Higher absorbance values indicate greater reducing ability.

Assay of Superoxide Radical Scavenging Activity

Superoxide radicals were generated in the system of pyrogallol autoxidation in an alkalescent condition [20]. A 4.5-ml volume of 0.05 M Tris–HCl (pH 8.2) was incubated at 25 °C for 25 min, then 1 ml of EPS-1 and EPS-2 at different concentrations (0.15, 0.30, 0.6, 1.25, 2.5, and 5 mg/ml) were added along with 0.4 ml of 2.5 mmol pyrogallol, mixed, and reacted for 5 min. Finally, the absorbance of reaction mixture at 299 nm was measured. Vitamin C (Vc) was used as a positive control, and H_2O was used as a negative control. The superoxide radical scavenging effect (%) was calculated: $(A_0–A_1)/A_0 \times 100$ %, where A_0 is OD_{299} of negative control and A_1 is OD_{299} of treated EPS.

Assay of Hydroxyl Radical Scavenging Activity

Hydroxyl radicals were generated in the H_2O_2–$FeSO_4$ system and were assayed by the salicylic acid method. A 2 ml of EPS-1 and EPS-2 with different concentrations (0.15, 0.30, 0.6, 1.25, 2.5, and 5 mg/ml) were mixed with 2 ml of 6 mmol/l $FeSO_4$ and 2 ml of 6 mmol/l H_2O_2. The change in absorbance caused by the color change of salicylic acid was measured at 510 nm. Vitamin C (Vc) was used as a positive control, and H_2O was used as a negative control. The hydroxyl radical scavenging effect (%) was calculated: $(A_0–A_1)/A_0 \times 100$ %, where A_0 is OD_{510} of negative control and A_1 is OD_{510} of treated EPS.

Measurement of Intracellular ROS in HepG2 Cells

Intracellular ROS was estimated with a fluorescent probe, DCFH-DA [1]. The HepG2 cells were pretreated with EPS-1 and EPS-2 with final concentrations of 100 μg/ml for 12 h. Then, hydrogen peroxide (H_2O_2) at the final concentration of 100 μmol/l was added to each well at 0, 2, and 4 h. After incubation, cells were incubated with DCFH-DA (10 μmol/l) diluted in serum-free culture medium for 20 min at 37 °C. Imaging in live cells was performed in cover glass with an inverted confocal microscope with a 10 × objective (LSM510 NLO, Zeiss). The fluorescence was induced with excitation at 485 nm and monitored at 520 nm.

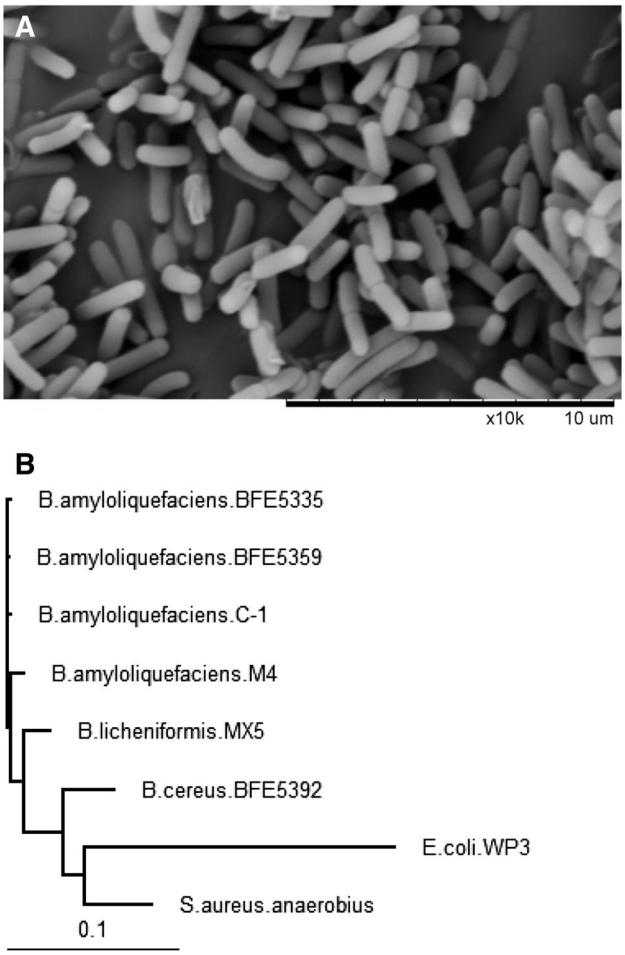

Fig. 1 Scanning electron micrograph (**a**) of C-1 grown on LB medium with 1 % glucose at 30 °C for 72 h. *Bar*, 10 μm; magnification, ×10,000; neighbor-joining tree showing the phylogenetic relationships of *B. amyloliquefaciens* C-1 to other *Bacillus* species (based on the 16S rRNA sequence) analyzed by CLUSTAL W. Sequence accession number in GenBank: *B. amyloliquefaciens* M4 (JX036449), *B. amyloliquefaciens* BFE5335 (GU250447), *B. amyloliquefaciens* BFE 5359 (GU250449), *B. licheniformis* MX5 (JX027378), *B. cereus* BFE5392 (GU250443), *E. coli* WP3 (JQ993870), *S. aureus* subsp. anaerobius (D83355)

Effect of EPSs on Malondialdehyde (MDA) and Speroxide Dismutase (SOD) Activity in H_2O_2-Induced HepG2 Cells

After pretreatment with EPS-1 and EPS-2 for 12 h, HepG2 cells were suspended in an appropriate volume of lysis

buffer (50 mmol/l Tris–HCl, pH 8.0, 50 mmol/l EDTANa_2, 0.2 mol/l NaCl, 1 % Triton X-100), and the yielded cell homogenate was immediately centrifuged at 10,000*g* for 15 min 4 °C. The supernatant was stored at −20 °C prior to the assays. SOD activity and MDA were determined spectrophotometrically using commercially available assay kits (Nanjing Jiancheng, CN) as described previously [17].

Statistical Analysis

The data were analyzed by ANOVA, and $P < 0.05$ was selected prior to the experiments to reflect statistical significance. Unless otherwise stated, all results are expressed as the mean ± SD ($n \geq 3$). All the analyses were conducted using the General Linear Model (GLM) procedure of SAS Version 9.1.3 (SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Characterization and Identification of Isolated Strain C-1

Observed under SEM, strain C-1 is a rod-shaped bacterium (0.6–0.8 μm in diameter and 2 μm in length, Fig. 1a). And the colonies showed sticky, translucent, white, and mucoid appearance (5 mm in diameter after 3 days on LB medium with 1 % glucose at 30 °C). The strain grew aerobically from 25 to 50 °C and its optimal temperature occurred at 30 °C. Comparison of 16S rRNA gene sequence among C-1 (1433 bp, accession no. JX028840) and other 7 bacterials from GenBank by BLAST analysis, the result showed the closest strains (99 % similarity) were *B. amyloliquefaciens* BFE 5335 (GU250447), *B. amyloliquefaciens* BFE 5359 (GU250449), and *B. amyloliquefaciens* M4 (JX036449). From the N-J tree (Fig. 1b), strain C-1 was identified as *B. amyloliquefaciens*.

Kinetics of Growth and Isolation of EPSs

As a major class of natural products, metabolites from microorganisms are one of the most reproducible, dependable, and stable source in development of food additives, bio-drugs, bio-active compounds. Among *Bacillus* species, strain of *B. amyloliquefaciens* is widely distributed in soil and considered as a useful bacterium in industry processes, as it is able to produce enzyme proteins and other useful products. The sticky characteristics of C-1 colonies indicated that *B. amyloliquefaciens* C-1 was able to produce metabolites consisting mainly of EPSs. The EPS produced by C-1 during the whole fermentation period is illustrated in Fig. 2a. This strain exhibited an exponential

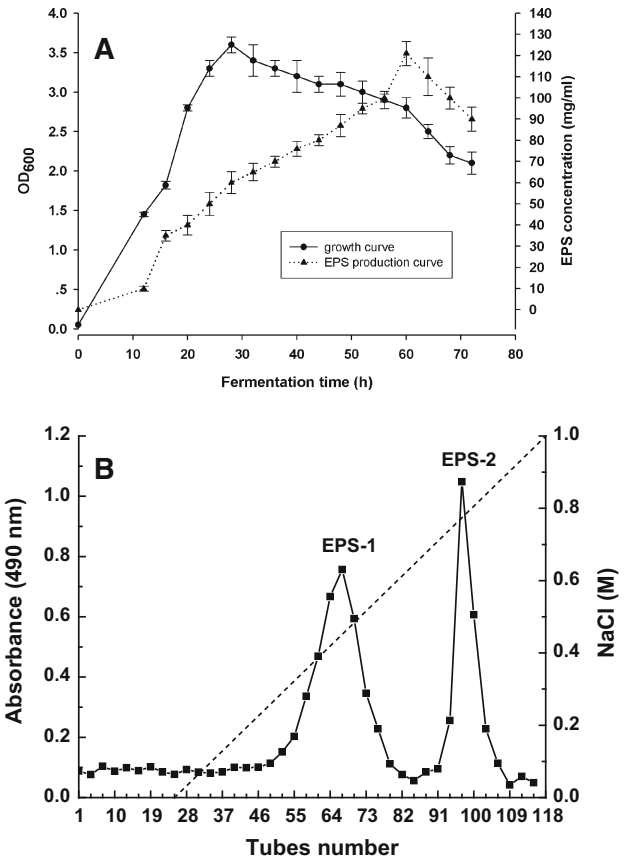

Fig. 2 Kinetics of growth and EPS production (**a**) of *B. amyloliquefaciens* C-1 in batch cultures showing the bacterial cell counts (*filled circle*) and amounts of EPS produced (*filled triangle*). Each value represents the average of triplicate measurements; chromatography of eluted crude EPS on DEAE-cellulose column (**b**)

growth after incubation for 12 h and then entered stationary phase at 28 h, with cells having optical density at 600 nm of approximately 3.65. The EPS production increased gradually along with bacterial growth in LB medium with 1 % glucose (initial pH 7.5, 30 °C). The maximum OD_{600} of C-1 culture growth was 3.65 at 28 h, while the maximum EPS production was 121 mg/l at 60 h, which was the late stationary growth with OD_{600} of 2.76. It agrees with the point that EPS-producing microorganisms usually reach their optimal growth within the initial 24 h of incubation, whereas maximal EPS production occurs in later stages of growth [20]. Then the amount of EPS decreased probably due to the action of glycohydrolases in the culture that catalyzed the degradation of polysaccharides [8].

Isolation and Purification of EPSs

Under optimal condition of growth, crude EPS was isolated from fermentation broth of *B. amyloliquefaciens* C-1 by ethanol precipitation, deproteinization, dialyzation, and

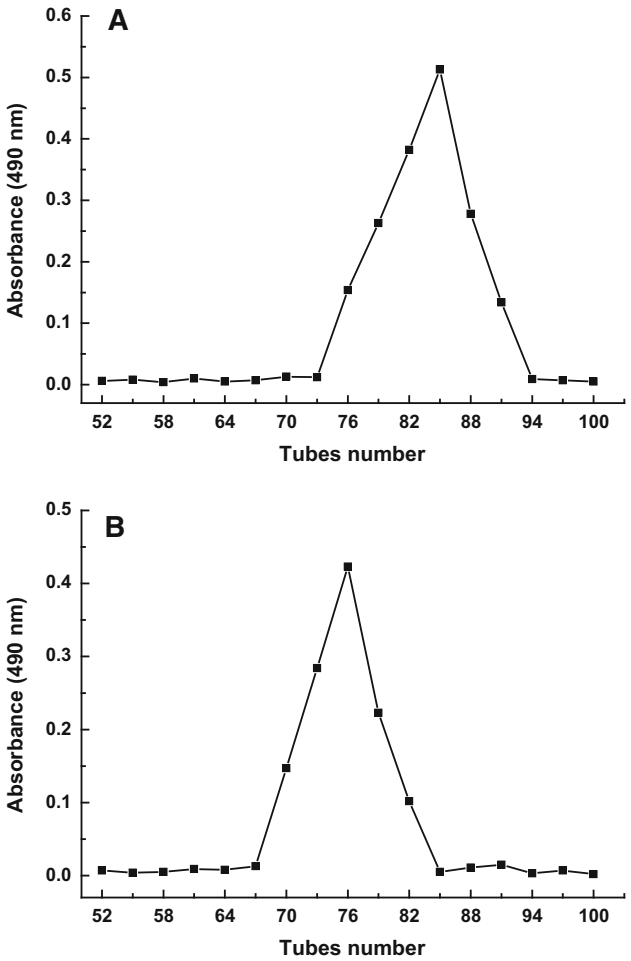

Fig. 3 Elution profile of chromatography of EPS-1 (**a**) and EPS-2 (**b**) produced by *B. amyloliquefaciens* C-1 on sepharose CL-6B gel filtration column

dryness. The extraction yield of crude EPS was approximately 12.1 % of the whole fermentation broth. The crude EPS was purified using anion-exchange chromatography with NaCl (0–1.0 M) gradient elution (Fig. 2b). This elution profile of crude EPS showed two relatively symmetrical peaks, indicating their homogeneity. It is shown that this crude EPS has two major water-soluble polysaccharides, named EPS-1 (tube49–82) and EPS-2 (tube91–109), and the extraction yields of the two components were 40.2 and 33.9 %, respectively. At the same time, absorbance at 280 nm was also determined, but no significant absorbance was shown, indicating these EPSs were free of any peptide chains.

Each polysaccharide was further purified through sepharose CL-6B column and eluted with 0.9 % NaCl (Fig. 3a, b). It showed that EPS-1 and EPS-2 only had a single peak profile, indicating a purified polysaccharide. Extraction yields of EPS-1 and EPS-2 reached up to 57.1 and 62.5 %, respectively. The fraction was collected separately, dialyzed, and lyophilized for further characterization.

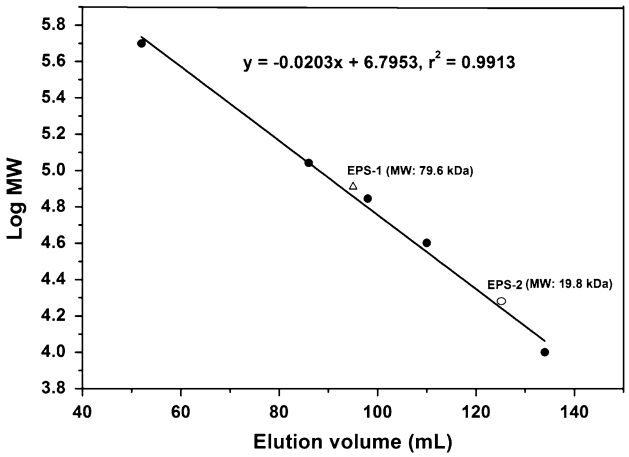

Fig. 4 Plot of log molecular weight versus elution tubes for various dextran standards (500, 100, 80, 70, 40, and 10 kDa). EPS-1 (*triangle*) and EPS-2 (*open circle*) is estimated to have a molecular weight of 79.6 and 19.8 kDa ($n = 3$), respectively.

Molecular Weight (MW) and Monosaccharide Compositions of EPS-1 and EPS-2

To characterize the structures of the EPS-1 and EPS-2, the apparent average molecular weight of EPS-1 and EPS-2 were determined by gel permeation chromatography as shown in Fig. 4. The calibration curve for MW determination was made using a series of β-glucan standards: lg MW $= -0.0203x + 6.7953$ ($r^2 = 0.9913$), where x is elution volume. Based on the calibration curve, the MW of EPS-1 and EPS-2 were 79.6 and 19.8 kDa, respectively. The monosaccharide compositions of EPS-1 and EPS-2 were analyzed by gas chromatography, and the result was shown in Table 1. It revealed that the monosaccharide contents were different for the two polysaccharides. In general, glucose was the most abundant monosaccharide in both EPS-1 and EPS-2. EPS-1 was comprised of D-glucose, D-mannose, D-galactose, and D-arabinose in the molar ratio of 15:4:2:1, respectively. Several reports explained that galactose and arabinose are associated with the antioxidant activities of polysaccharides [5, 28]. However, the EPS-2 was comprised of D-glucose and D-mannose in a 3:1 ratio. It may be an important factor of EPS-2 that EPS-2 would have different antioxidant activity compared with EPS-1 in our further assays. EPS from *Streptococcus macedonicus* Sc136 was composed of D-glucose, D-galactose, and *N*-acetyl-D-glucosamine [27]. Another EPS produced from *V. harveyi* strain VB23 was composed primarily of D-galactose and D-glucose [4]. All the results indicated that the monosaccharide compositions were quite different from different bacterial cultures.

Table 1 Chemical composition (%, dry weight) of EPS-1 and EPS-2 produced by *Bacillus amyloliquefaciens* strain C-1

Polysaccharide	Composition of polysaccharide (%)			
	Glucose	Mannose	Galactose	Arabinose
EPS-1	68.31 ± 3.42	17.85 ± 0.89	9.27 ± 0.46	4.57 ± 0.23
EPS-2	73.82 ± 3.70	26.18 ± 1.31	nd	nd

nd not detected

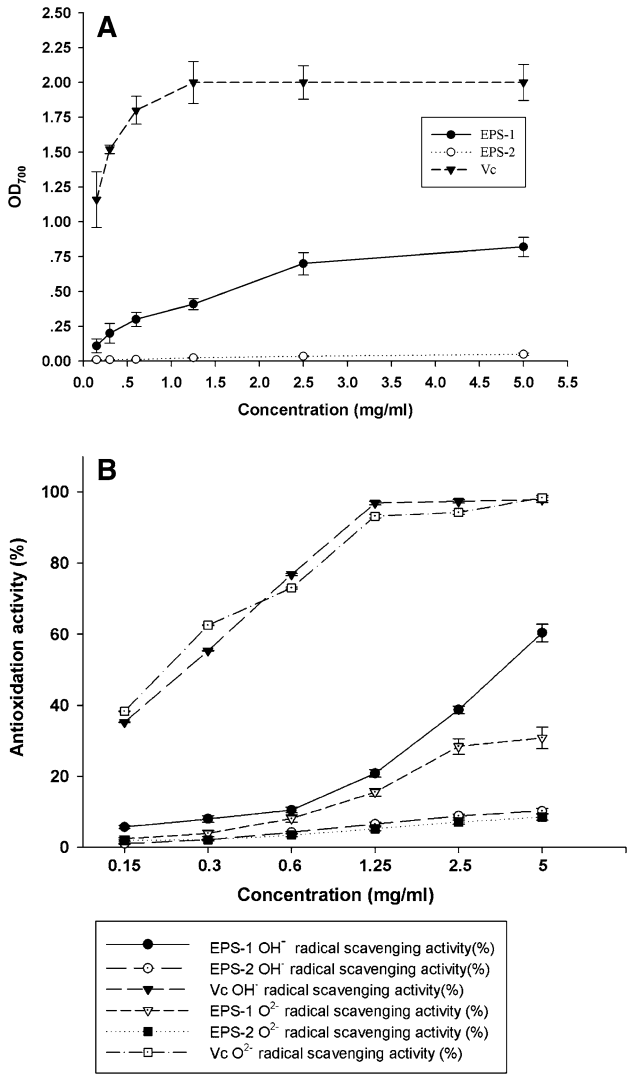

Fig. 5 Antioxidant activity of EPS-1 and EPS-2 produced by *B. amyloliquefaciens* strain C-1. The reducing power (**a**), and the OH^- scavenging and O^{2-} scavenging activities (**b**)

Antioxidant Activity of EPSs In Vitro

For measuring antioxidant property of EPS-1 and EPS-2 from strain C-1, different methods including reductive power assay, superoxide radicals, and hydroxyl radical scavenging assays have been used corresponding to different levels of antioxidant action. The reducing power of a compound may serve as a significant indicator of its potential antioxidant activity [14]. As shown in Fig. 5a, EPS-2 showed no obvious reductive ability when compared with Vc. While the EPS-1 showed significant reducing power in dose-dependent manner, suggesting a high potential in hydrogen-donating ability which could react with free radicals to convert them into more stable products and thereby terminate radical chain reactions [10]. However, none of the EPS-1 samples had a reductive activity approaching that of the same concentration of Vc. At 5 mg/ml EPS-1, its reducing power (0.82) was less than half of that of Vc (2.1).

Superoxide radicals are harmful free radicals for cellular components, and the presence of superoxide anions can magnify cellular damages, as they produce other types of free radicals and oxidizing agents [3]. Therefore, the superoxide radical scavenging ability is of great importance to its potential antioxidant activity. The scavenging activities of superoxide radical by EPSs from strain C-1 are shown to be dose-dependent in Fig. 5b. The superoxide radical scavenging effect of C-1 EPS-1 ranged from 2.4 % at 0.15 mg/ml to 30.8 % at 5 mg/ml, which was similar to that of *Cordyceps militaris* SU5-08, *Bacillus edudis* [16]. And for EPS-2, little superoxide radicals scavenging activity was found. And the scavenging effect ranged from 1.9 % at 0.15 mg/ml to 8.5 % at 5 mg/ml, respectively. It has been reported that the mechanism of superoxide anion scavenging may be associated with the dissociation energy of O–H bonds [24].

Hydroxyl free radicals and their derivative radicals are highly potent oxidants, which can react with most biomacromolecules in living cells and induce severe biological damage and lipid peroxidation. As shown in Fig. 5b, the EPS-1 had an obvious hydroxyl radical scavenging activity from 6 % increased to 60.4 % in a dose-dependent manner, which was higher than that of 50.8 % for *Boletus edudis*, 49.4 % for *Pholiota adipose*, 26.2 % for *Antrodia camphorate* at 5 mg/ml, respectively [16]. It has been proposed that the hydrogen or electron abstraction mechanism might be the best explanation of why polysaccharides can inhibit the formation of hydroxyl radicals [6, 26]. While for EPS-2, those scavenging activity was less than 10 % of EPS-1 activity at the same concentration.

Multiple mechanisms account for the antioxidant activity of different compounds, including prevention of

chain initiation, binding to transition metal ion catalysts, decomposition of peroxides, and prevention of scavenging ability [17]. Among the biological characteristics and functions studied above, the results revealed that although there were 2 EPS fractions purified from C-1 culture, the functional EPS was only EPS-1, which exhibited good antioxidant activity in vitro for superoxide radicals, hydroxyl radical scavenging activity, and reductive power (Fig. 5). It might be attributed to the functional groups in the EPS fraction, which can donate electrons to reduce the radicals to a more stable form or react with the free radicals to terminate the radical chain reaction [15]. These characteristics are similar to those of EPS fractions from *Bifidobacterium animalis* [29] and *Paenibacillus polymyxa* [18]. For EPS-2, the function was still unknown. Maybe it contributes to the anti-tumor activity, immunostimulatory, anti-microbial activity which was shown in crude C-1 EPS [19].

Effects of EPSs on Intracellular ROS, MDA and SOD in H_2O_2-Treated HepG2 Cells

According to the results above, the effects of antioxidant activities of EPS-1 exhibit strong free radical scavenging effects, whereas EPS-2 nearly had no antioxidant activities. In order to investigate in-depth the antioxidant activities of the EPS-1 and EPS-2, protective effects of EPS-1 and EPS-2 on HepG2 cells against H_2O_2 injury were evaluated. Figure 6a showed that pretreated cells with EPS-1 for 2 and 4 h significantly decreased the fluorescence of ROS induced by H_2O_2. Particularly, the decreased fluorescence with increased time indicated that the inhibition of ROS by EPS-1 is time-dependent. Compared with the EPS-1, EPS-2 nearly did not inhibit the formation of intracellular ROS ($P > 0.01$). MDA is often used as a marker of the lipid peroxidation being consequently an indicator of oxidative damage in cell membranes. As shown in Fig. 6b, when treated with 500 μg/ml EPS-1, the intracellular MDA levels were significantly decreased ($P < 0.05$) compared with those cells treated by H_2O_2, indicating that EPS-1 could significantly improve the antioxidant status by resisting the lipid peroxidation in H_2O_2-treated HepG2 cells. However, no significant differences in the ability of EPS-2 (500 μg/ml) to inhibit MDA ($P > 0.01$) were observed. Finally, the effects of EPS-1 and EPS-2 on the H_2O_2-induced changes of SOD activity in HepG2 cells were also investigated. As shown in Fig. 6c, H_2O_2 treatment decreased intracellular SOD activity compared with control cells ($P < 0.05$); however, pretreatments of EPS-1 for 12 h could restore SOD activity of H_2O_2-treated HUVECs. In contrast, the EPS-2 showed the similar decresed level of SOD activities as the H_2O_2 treated control. These findings further indicated that only EPS-1 could

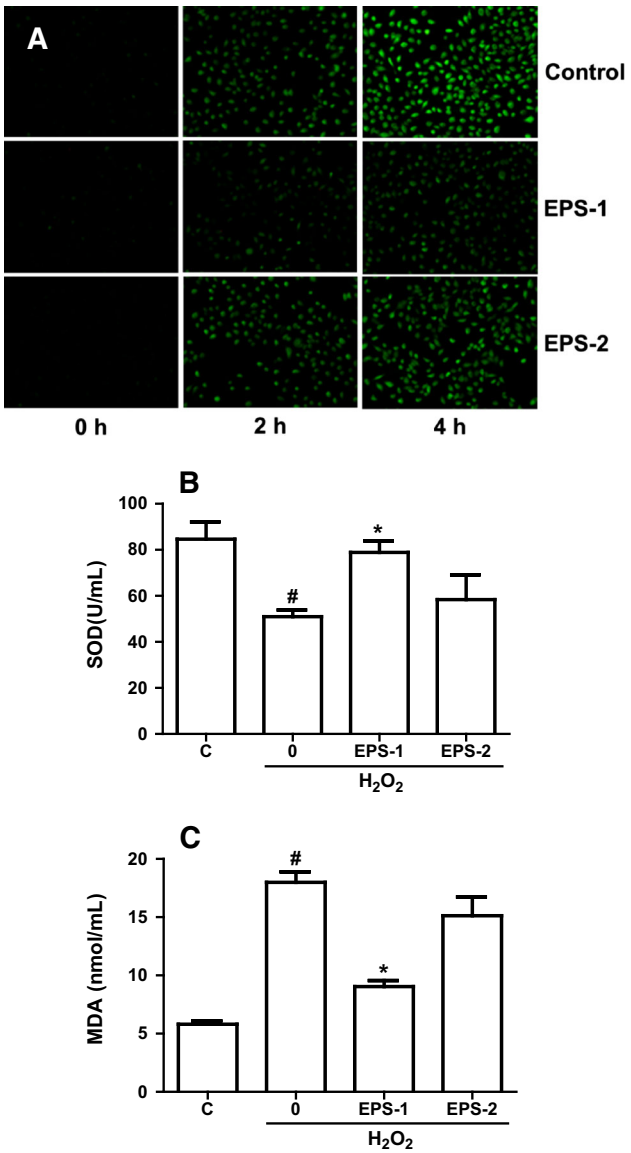

Fig. 6 Antioxidant activities assays in HepG2 cells which was treated with EPS-1 and EPS-2 of *B. amyloliquefaciens* C-1. ROS production determined by confocal microscope using the dichlorofluorescein (DCF) (magnification, ×100) (**a**), SOD activity analysis (**b**), and MDA analysis (**c**) in HepG2 cells with H_2O_2-stimulation. Results are presented as means ± SDs

significantly improve the antioxidant status in H_2O_2-treated HepG2 cells, which is consistent with the antioxidant activities such as reducing ability and scavenging superoxide radical ability. Many EPSs were reported to be potential antioxidants secreted by bacteria and fungus. For example, EPS from mycelial culture of *Cordyceps sinensis* fungus Cs-HK1 displayed moderate antioxidant activities with a trolox equivalent antioxidant capacity and a ferric reducing ability of plasma [15]. EPS from *L. plantarum* C88 inhibited the formation of MDA and raised the activities of SOD and total antioxidant capacities (T-AOC)

in Caco-2 cells [33]. But it is currently unclear how polysaccharides affect the intracellular antioxidant system. EPS-1 responded to H_2O_2-induced oxidative stress by increasing enzymatic (such as SOD) and non-enzymatic (such as MDA) intracellular antioxidant defenses, and it probably could be the mechanism of the protective effect of EPS-1 produced by *B. amyloliquefaciens* C-1 on H_2O_2-induced oxidative damage in HepG2 cells. It may protect bacterial cells from desiccation, bacteriophage attack, and phagocytosis. The great differences existed in antioxidant activities of EPS-1 and EPS-2 possibly depended on their monosaccharide composition, type of linkages and chain conformation. Therefore, the beneficial health effects of EPS-1 produced by *B. amyloliquefaciens* C-1 might be associated with its prominent antioxidant activities.

Conclusion

In summary, EPS-1 and EPS-2 were isolated with high purity and identified as typical polysaccharides from *B. amyloliquefaciens* C-1. EPS-1 was composed of glucose, mannose, galactose, and arabinose in a ratio of 15:4:2:1 with molecular weight of 79.6 kDa, while EPS-2 was composed of glucose and mannose in a ratio of 3:1 with molecular weight of 19.8 kDa. EPS-1 exhibited strong antioxidant activity by quenching hydroxyl and superoxide anion radicals in vitro and in HepG2 cells against H_2O_2-induced injury. However, no significant antioxidant activities of EPS-2 were observed. Therefore, it is suggested that EPS-1, but not EPS-2 might provide a source of natural antioxidants with potential value for functional foods or therapeutics. Further works should be done for detailed structural characterization of EPSs and the corresponding relationships between their structures and functionalities.

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Conflict of interest The authors declare that they have no competing interests to this paper.

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