Carbohydrate Polymers 80 (2010) 908–914

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Antioxidant activity of an exopolysaccharide purified from *Lactococcus lactis* subsp. *lactis* 12

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ARTICLE INFO

Article history:
Received 10 December 2009
Received in revised form 23 December 2009
Accepted 7 January 2010
Available online 11 January 2010

Keywords:
Lactococcus lactis subsp. *lactis* 12
Exopolysaccharide
Isolation and purification
Antioxidation activities

ABSTRACT

A novel exopolysaccharide (EPS) was obtained by ultra-filtration, ion exchange and sizing chromatography from a culture of *Lactococcus lactis* subsp. *lactis* 12. The EPS was mainly composed of fructose and rhamnose with a mean molecular weight of 6.9×10^5 Da. The EPS and its antioxidant properties were evaluated *in vitro* and *in vivo*. The EPS displayed strong antioxidant effects, exhibited the ability to scavenge hydroxyl and superoxide anion radicals, and significantly decreased the level of malondialdehyde (MDA), even while increasing the activity of catalase (CAT) and superoxide dismutase (SOD) in mice in a dose-dependent manner. The results suggest that the EPS has direct and potent antioxidant properties.

1. Introduction

Reactive oxygen species (ROS), such as hydroxyl and superoxide anion radicals, are highly reactive molecules derived from the metabolism of oxygen. They are often byproducts of biological reactions. *In vivo*, some ROS play positive roles in cell physiology, but they may also damage cell membranes and DNA, inducing oxidation that causes membrane lipid peroxidation and decreased membrane fluidity (Finkel & Holbrook, 2000; Melov et al., 2000). In recent years, many studies have shown that ROS are responsible for various conditions and diseases, including cancer, Alzheimer's disease, Parkinson's disease, epilepsy, inflammation, retrolental fibroplasias, atherosclerosis, lung injury, and ischemia reperfusion injury (Raouf, Patrice, Andre, Jean-Michel, & Yvan, 2000).

Although almost all organisms possess antioxidant defense and repair systems, these systems are unable to prevent entirely the damage caused by ROS (Ke et al., 2009). Therefore, exogenous antioxidants are frequently added to food to aid preservation, but also for their health benefits. However, many synthetic antioxidants used in foods, such as butylated hydroxyanisole and butylated hydroxytoluene, may exhibit cytotoxicity (Grice, 1988; Luo & Fang, 2008; Valentao et al., 2002). For this reason, more attention has been paid to natural non-toxic antioxidants in an effort to protect the human body from free radicals and retard the progress of many chronic diseases.

Polysaccharides widely occur in plants, microorganisms (fungi and bacteria), algae and animals. Due to their ready availability and rheological properties, polysaccharides represent a class of high-value polymers with many industrial applications in the food, cosmetic, textile, and pharmaceutical industries. One such application is their use as emulsifiers, stabilizers and texture enhancers in the food industry. In recent years, an increasing body of evidence suggests that some polysaccharides isolated from cultivable microbial sources have antioxidant capabilities and low cytotoxicity (Krizkova et al., 2006; McCue & Shetty, 2002). Many papers have been published discussing the screening, isolation, characterization, biosynthesis, and functional properties of the EPS produced by lactic acid bacteria (LAB) (Maeda, Zhu, Suzuki, Suzuki, & Kitamura, 2004; Shene & Bravo, 2007; Shene, Canquil, Bravo, & Rubilar, 2008; Vinderola, Perdigon, Duarte, Farnworth, & Matar, 2006). There have been reports of EPS produced by some strains of *Lactococcus lactis*, such as *L. lactis* subsp. *cremoris*. However, for strains of *L. lactis* subsp. *lactis*, no published study has discussed the production, purification, characterization, and biological properties of EPS in relation to its antioxidant properties *in vitro* and *in vivo* (Ayala-Hernandez, Hassan, Goff, & Corredig, 2009; Ayala-Hernandez, Hassan, Goff, Mira de Orduna, & Corredig, 2008; Ruas-Madiedo, Tuinier, Kanning, & Zoon, 2002).

In this paper, we describe the production, isolation, purification, characterization and antioxidant potential of EPS derived from *L. lactis* subsp. *lactis* 12 isolated from Chinese pickled cabbage.

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doi:10.1016/j.carbpol.2010.01.005

2. Materials and methods

2.1. Reagents and materials

Ascorbic acid (vitamin C), hydrogen peroxide (H_2O_2), ferrous sulfate ($FeSO_4$), trichloroacetic acid (TCA) and brilliant green were purchased from Nanjing Chemical Reagent Company, Nanjing, PR China. Ultra-filtration membranes were purchased from Shanghai Mosu Company, Shanghai, PR China. Culture media, DEAE-cellulose, Sepharose CL-6B, Sephacryl S-300 HR, and standard dextrans (2000, 845, 482, 133, 70, 40 and 10 kDa) were purchased from Pharmacia Biotech, Sweden. Reduced nicotinamide adenine dinucleotide (NADH), nitroblue tetrazolium (NBT), phenazine methosulfate (PMS), and standard monosaccharides (D-xylose, D-galactose, D-glucose, D-fructose, L-rhamnose and D-mannose) were purchased from Sigma Chemical Co., St. Louis, MO, USA. The assay kits for SOD, CAT and MDA were purchased from Jiancheng Bioengineering Institute, Nanjing, PR China. All other chemicals used were ultra pure or analytical grade purchased from Nanjing Chemical Reagent Company, Nanjing, PR China.

2.2. Production, isolation and purification of EPS

2.2.1. Production of EPS

Lactococcus lactis subsp. *lactis* 12 isolated from Chinese pickled cabbage was conserved by the Dairy Biotechnology Institute, Nanjing Normal University. The do Man-Rogosa-Sharpe (MRS) agar medium was used to ferment *L. lactis* subsp. *lactis* 12 to harvest EPS. The medium was autoclaved at 121 °C for 15 min. The fermentation temperature, medium initial pH value, inoculation proportion, and fermentation time were 35 °C, 6.0, 3.0% (v/v), and 24 h, respectively.

2.3. Isolation and purification of EPS

2.3.1. Ultra-filtration

Grown culture was centrifuged (6000 rpm, 10 min, 4 °C) to eliminate the biomass. Cell-free culture was ultra-filtrated using a hollow-fiber membrane with a molecular weight cutoff of 100 kU (obtained from Shanghai Mosutech Co. Ltd., PR China), at an operating temperature of 25 °C and pressure of 0.08 MPa. The retentate solution obtained was collected as EPS broth. A phenol–sulfuric acid method was used to analyze the EPS (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

2.3.2. Isolation and purification of EPS

The proteins in the EPS broth were removed using 10.0% (v/v) TCA and 6000 rpm centrifugation for 30 min at 4 °C. The EPS, which was in supernatant, was precipitated from the broth by the addition of 90% (v/v) cold ethanol three times, and left overnight at 4 °C. The resulting precipitate was collected by centrifugation at 6000 rpm for 30 min at 4 °C, re-dissolved in distilled water and dialyzed (MWCO 6000-8000) using distilled water for 24 h at 4 °C. The dialyzed solution was concentrated and used as crude EPS solution for further purification.

The crude EPS solution was subjected to a DEAE–cellulose anion-exchange chromatography column (2.6 cm × 50 cm) pre-equilibrated with distilled water (pH 7.0), eluted with a linear gradient of 0–1 M NaCl in distilled water with a flow rate of 30 mL/h, and monitored using a refractive index RID-10A detector (Shimadzu, Tokyo, Japan). The fractions containing EPS were pooled and the total carbohydrate content was quantified using a phenol–sulphuric acid method. Protein concentrations were estimated using the method outlined by Lowry with bovine serum albumin as the standard (Lowry, Rosebrough, Farr, & Randall, 1951). The obtained EPS solution was dialyzed against distilled water, concentrated and re-fractionated over a Sepharose CL-6B column (2.6 cm × 50 cm) and then a Sephacryl S-300 column (2.6 cm × 50 cm) pre-equilibrated with distilled water, eluted with a linear gradient of 0–1 M NaCl in distilled water at a flow rate of 24 mL/h, and monitored using a refractive index RID-10A detector (Shimadzu, Tokyo, Japan). The EPS fractions were pooled, dialyzed against distilled water, concentrated, and lyophilized. The dried EPS powder was called EPS-I.

2.4. Analysis of EPS-I

2.4.1. Monosaccharide composition and molecular weight analysis of EPS-I

The monosaccharide composition of EPS-I was analyzed using thin-layer chromatography (TLC) performed on pre-coated aluminum silica gel plates (GF254, Merck) (Yan, Guo, Li, Wu, & Gou, 2006). EPS-I was hydrolyzed in 12 M HCl at 121 °C in an autoclave for 4 h followed by using NaOH to adjust the pH to neutral before dialysis. D-Xylose, D-galactose, D-glucose, D-fructose, L-rhamnose, and D-mannose were used as monosaccharide standards against the EPS-I hydrolyzate. A mixed solution composed of ethylacetate, pyridine, alcohol, and water in a ratio of $C_4H_8O_2$:C_5H_5N:C_2H_6O:H_2O = 8:1:1:2 by volume was used as an extending system. The change of spot colors on the chromatograms was detected by spraying a mixed solution of 2% (v/v) aniline, 2% (w/v) diphenylamine dissolved in acetone, and 85% (v/v) phosphoric acid in a ratio of 5:5:1 by volume. Qualitative results were obtained in accordance with the Rf value of the standard spots.

Size-exclusion chromatography for the purified EPS-I was performed using a Sephacryl S-300 HR (50 × 1.5 cm) with an AKTA Purifier system (Amersham Pharmacia Biotech, Sweden) and 0.2 M NaCl as eluent to determine the molecular weight of EPS-I. Preliminary calibration of the column was conducted using dextrans of different molecular weights (2000, 845, 482, 133, 70, 40 and 10 kDa). The molecular weight was calculated based on the calibration curve obtained by using various standard dextrans (Yamamoto, Nunome, Yamauchi, Kato, & Sone, 1995).

2.4.2. UV and IR analysis of EPS-I

UV–vis absorption spectra were recorded using a Shimadzu MPS-2000 spectrophotometer between 190 and 290 nm. The infrared (IR) spectra of the EPS-I were determined using a Fourier transform infrared spectrophotometer (Nexus 5 DXC FTIR, Thermo Nicolet, Madison, WI, USA). The purified EPS-I was ground with spectroscopic-grade potassium bromide (KBr) powder and then pressed into 1 mm pellets for FTIR measurement in the 4000–500 cm^{-1} frequency range (Zhbankov, Adnanov, & Marchewka, 1997).

2.5. Antioxidant activities of EPS-I in vitro

2.5.1. Total antioxidant capacity assay of EPS-I

Suitable working standards (0.24, 1.0, 5.0, and 10 mg/mL) were prepared by dissolving the EPS-I in distilled water. Aliquots (0.30 mL) of the sample were mixed with 3 mL of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped with aluminum foil and incubated at 95 °C for 90 min (Umamaheswari & Chatterjee, 2008). The tubes were cooled to room temperature and absorbance was measured at 695 nm against a blank. Ascorbic acid was used as a standard. Total antioxidant capacity was expressed as equivalents of ascorbic acid (Raghavan et al., 2003).

2.5.2. Assay of EPS-I's superoxide anion scavenging activity

The assay was based on the sample's capacity to inhibit the photochemical reduction of NBT in the NADH–NBT–PMS system (Kanatt, Chander, & Sharma, 2007). The reaction mixture consisted

of 1.0 mL of NBT (78 μM in 20 mM potassium phosphate buffer pH 7.4), 1.0 mL of NADH (468 μM in 20 mM potassium phosphate buffer pH 7.4) and 1.0 mL of an appropriate sample solution. The reaction was initiated by addition of 0.4 mL of PMS (60 μM in 20 mM potassium phosphate buffer pH 7.4) to the mixture. The tube was incubated at 37 °C for 5 min and absorbance was measured at 560 nm against a blank. Decreased absorbance of the reaction mixture indicated increasing superoxide anion scavenging activity. Ascorbic acid was used as a positive control, and a control containing all of the reaction reagents except the samples was prepared. The superoxide radical effect was calculated as scavenging activity (%) = (1 − absorbance of sample/absorbance of control) × 100%.

2.5.3. Assay of EPS-I's hydroxyl radical scavenging activity

The hydroxyl radical system generated by a Fenton reaction was used to evaluate EPS-I's hydroxyl radical scavenging activity (He, Luo, Cao, & Cui, 2004). Briefly, the reaction mixture contained 1.0 mL of brilliant green (0.435 mM), 2.0 mL of $FeSO_4$ (0.5 mM), 1.5 mL of H_2O_2 (3.0%), and samples of various concentrations dissolved in distilled water. After incubation at room temperature for 20 min, absorbance was measured at 624 nm. The absorbance change of the reaction mixture indicated scavenging ability for hydroxyl radicals. Ascorbic acid was used as a positive control. Hydroxyl radical scavenging activity is expressed as:

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

where A_S is the absorbance in the presence of the sample, A_0 is the absorbance of the control in the absence of the sample, and A is the absorbance without the sample and Fenton reaction system.

2.6. Antioxidant capability of EPS-I in mice

2.6.1. Animal administration

Six-week-old male Kunming mice with body weights of 18–22 g (obtained from the Experimental Animal Center, Nanjing Medical University, PR China) were used. The mice were left to acclimatize for 1 week before the experiment. The animals were housed under a daily cycle of 12 h light and 12 h darkness at a temperature of 23 ± 1 °C and a relative humidity of 55 ± 5% throughout the experiment, with free access to commercial pellet food purchased from the Nanjing Qinglongshan Animal Resources Centre (PR China) and water. All the procedures were carried out in strict accordance with PR China legislation.

Mice were randomly divided into four groups of 8 mice each. Group 1 received 0.30 mL physiological saline and served as control mice. Group 2 received EPS-I doses of 40 mg/kg body weight and served as low-dose EPS-I treated mice. Group 3 received EPS-I doses of 80 mg/kg body weight and served as middle-dose EPS-I treated mice. Group 4 received EPS-I doses of 120 mg/kg body weight and served as high-dose EPS-I treated mice. EPS-I was dissolved in physiological saline according to the dosages and 0.30 mL of EPS-I solution was fed to mice by gastric intubation once a day throughout the experimental periods of 25 days. Twenty-four hours after the final administration, mice were anesthetized with ether and blood samples were obtained from the *arteria cervicalis* and centrifuged at 4000 rpm at 4 °C for 10 min to obtain the serums required for the measurement of CAT, SOD and MDA.

2.6.2. CAT, SOD and MDA assays

The activity levels of SOD, CAT and MDA were determined spectrophotometrically using commercially available assay kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, PR China) following the manufacturer's protocols.

CAT activity was determined by measuring the decrease in absorbance at 240 nm due to H_2O_2 decomposition. One unit of CAT activity was defined as the amount of decomposed H_2O_2 per second per milliliter of serum. CAT activity was expressed as U mL^{-1} serum.

SOD activity was determined spectrophotometrically at 550 nm by its ability to inhibit the oxidation of oxymine by the xanthine/xanthine oxidase system. One unit (U) of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the xanthine/xanthine oxidase system reaction. SOD activity was expressed as U mL^{-1} serum.

The level of lipid peroxidation was indicated by the amount of MDA in serum. A thiobarbituric acid reaction (TBAR) method was used to determine the amount of MDA. MDA content was expressed as nmol per milliliter of serum.

2.7. Statistical analysis

Tests were carried out in triplicate for all experiments. All the data was presented as means ± standard deviations (SD). Significant differences between treatments were tested by analysis of variance (ANOVA) followed by a comparison between treatments performed using Fisher's least significant difference (LSD) method, with levels of significance of $P < 0.05$ and $P < 0.01$.

3. Results and discussion

3.1. Isolation and purification of EPS-I

An EPS with a yield of 950.49 mg/L was obtained from fermented broth produced by *L. lactis* subsp. *lactis* 12. The density of the EPS was increased from 950.49 to 4790 mg/L with a purity of 70.38% and a recovery ratio of 95.52% via ultra-filtration using a membrane molecular weight cutoff of 100 kU at 25 °C and a pressure of 0.08 MPa.

The chromatographic elution profile for the crude polysaccharides in a DEAE–cellulose column is shown in Fig. 1A. The pooled fractions of peak I were concentrated and fractionated in a Sepharose CL-6B column. As shown in Fig. 1B, the sample was further separated into a main fraction of peak II and the remainder of the sample. The peak II fraction was collected, concentrated and re-fractionated in a Sephacryl S-300 column. As shown in Fig. 1C, only a fraction of peak III was obtained. The pooled fraction of peak III was close to homogeneity as indicated by a single and sharp peak in its elution profile, which was determined with size-exclusion chromatography using a Sephacryl S-300. The pooled fraction of peak III was called EPS-I and used for characterization and to analyze its antioxidant potential.

3.2. Analysis of EPS-I

According to the calibration curve using standard dextrans, the estimated equivalent dextran molecular weight of EPS-I was 6.9×10^5 Da.

There appeared two noticeable spots on the thin-layer chromatography for sugars released by acid hydrolysis of EPS-I. Their Rf values were 0.82 and 0.57, which showed that EPS-I is mainly composed of fructose and rhamnose. EPS-I showed no Rf values for D-xylose (0.72), D-galactose (0.45), D-glucose (0.52), or D-mannose (0.60).

An IR analysis of EPS-I is shown in Fig. 2. EPS-I displayed a negative response to the Bradford test and no absorption at 260 or 280 nm in the UV spectrum, indicating an absence of proteins and nucleic acid. As shown in Fig. 2, the bands in the 3411.66 cm^{-1} region are due to the hydroxyl-stretching vibration of EPS-I. The bands in the 2935.17 cm^{-1} region are due to C–H-stretching vibration, and the bands near 1655.10 cm^{-1} correspond to –OH bending vibration. The absorption at 1384.66 cm^{-1} is possibly due to symmetric CH_3 bending. The strong absorption at 1135.60 cm^{-1} was dominated by glycosidic linkage υ(C–O–C)-stretching vibration. Moreover, the

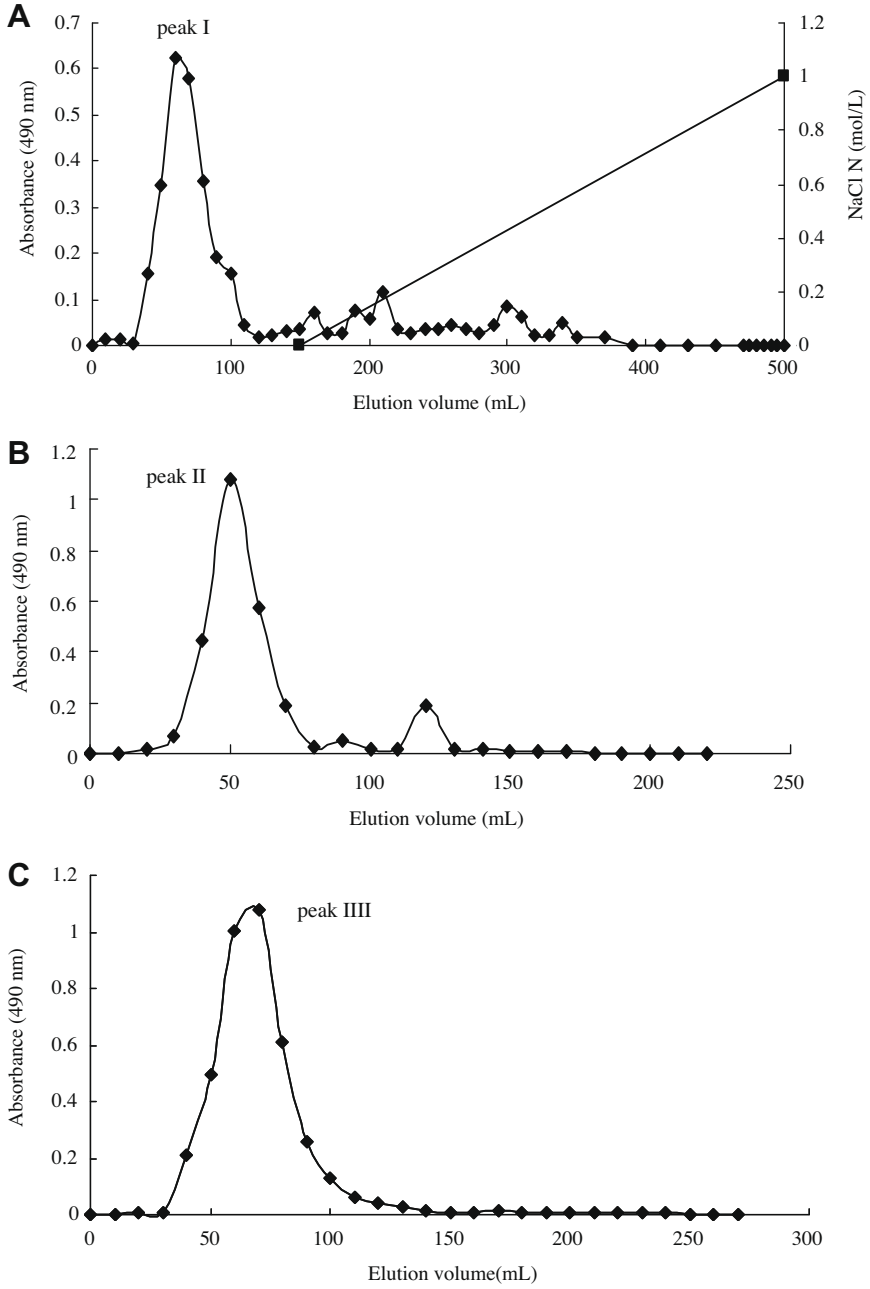

Fig. 1. Purification of EPS by DEAE–cellulose (A), Sepharose CL-6B (B) and Sephacryl HR 300 (C) chromatography.

characteristic absorptions at 872.30 cm^{-1} in the IR spectra indicated a β-anomeric configuration in EPS-I.

3.3. In vitro antioxidant activities of EPS-I

3.3.1. EPS-I's total antioxidant capacity

Total antioxidant capacity reflects the capacity of a non-enzymatic, antioxidant defense system. Fig. 3A represents the total antioxidant capacity of EPS-I at each concentration, expressed in terms of equivalents of ascorbic acid (a unit of ascorbic acid was 1 mg/mL). Antioxidant capacity increased markedly in a dose-dependent manner as the EPS-I concentration increased.

3.3.2. EPS-I superoxide anion scavenging activity

Superoxide anions are active free radical precursors that can react with biological macromolecules and cause tissue damage (Halliwell & Gutteridge, 1984). Superoxide anions play important roles in the formation of other ROS such as hydrogen peroxide, hydroxyl radical and singlet oxygen, which induce oxidative damage in lipids, proteins and DNA (Pietta, 2000; Wickens, 2001). Superoxide radicals were generated in the PMS/NADH system and assayed by reducing NBT. Fig. 3B illustrates the superoxide radical scavenging activity of 0.24, 1.0, 5.0 and 10.0 mg/mL of EPS-I compared to the same amounts of ascorbic acid. At all of the concentrations, the EPS-I samples exhibited varying degrees of antioxidant activity in a dose-dependent manner. The percentage inhibition is close to that of ascorbic acid at a dose of 10.0 mg/mL. Based on our results, it appears that EPS-I scavenges superoxide radicals by combining with superoxide radical ions to form stable radicals, thus terminating the radical chain reaction (Wang, Jonsdottir, & Olafsdottir, 2009).

3.3.3. EPS-I hydroxyl radical scavenging activity

Hydroxyl radicals are highly potent oxidants, which can react with biomolecules in living cells and cause severe damage (Gulcin,

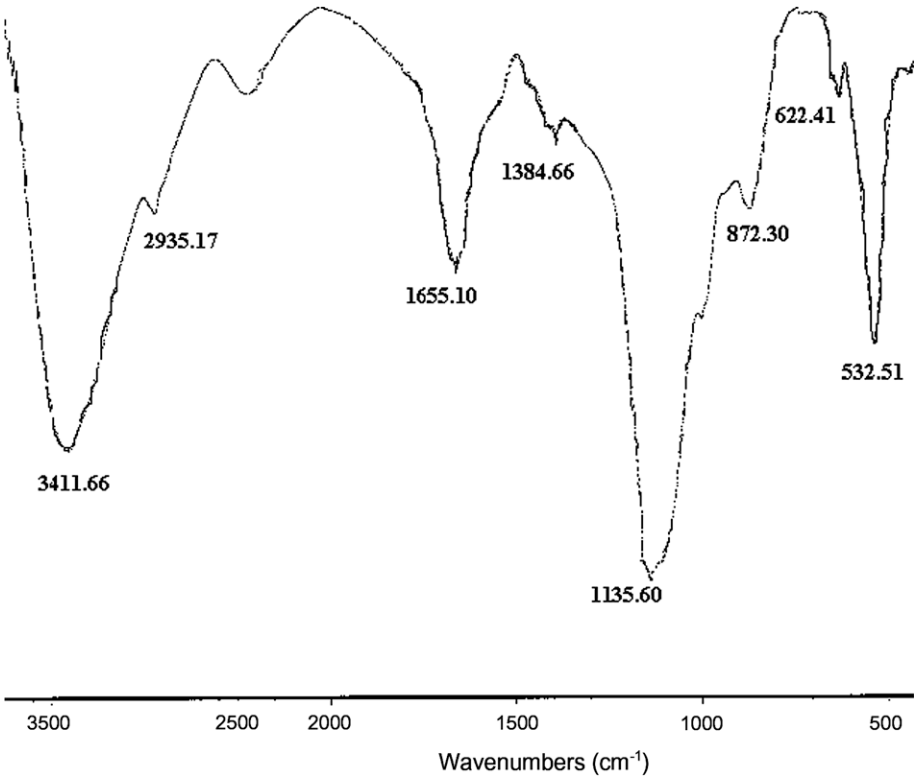

Fig. 2. EPS IR spectrogram.

2006). Fig. 3 shows that EPS-I and vitamin C both exhibited hydroxyl radical scavenging activity in a concentration-dependent manner. Vitamin C showed a slightly stronger effect on hydroxyl radicals than EPS-I under the same conditions. Studies of the antioxidant activity of various natural polysaccharides have suggested that hydroxyl radical scavenging activity was not due to scavenging directly but to the inhibition of the generation of hydroxyl radicals by chelating ions, such as Fe^{2+} and Cu^{2+} (Diplock, 1997; Ke et al., 2009; Simic, 1998). Metal-chelating activity may be one of the mechanisms by which EPS-I scavenges hydroxyl radicals, since such activity reduces the concentration of the catalyzing transition metal in lipid peroxidation. Among the transition metals, iron is the most important lipid oxidation prooxidant due to its high reactivity. The ferrous state of iron accelerates lipid oxidation by breaking down hydrogen and lipid peroxides to reactivate free radicals via the Fenton reaction ($Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH^{\cdot}$). Fe^{3+} ions also produce radicals from peroxides, although the rate is less than a tenth that of the Fe^{2+} ion. Thus, Fe^{2+} ion is the most powerful prooxidant among the various species of metal ions (Liu, Wang, Xu, & Wang, 2007).

3.4. EPS-I antioxidant activity in vivo

As shown in Table 1, administration of EPS-I significantly increased antioxidant enzyme (SOD and CAT) activity in serums compared with the control. The increase was dose dependent. The MDA level in serum, a main index of lipid peroxidation, was significantly decreased in all of the treated groups of mice compared with the control group. EPS-I inhibited MDA formation in serums more significantly as the dose increased from low through medium to high. These results suggest that EPS-I inhibits lipid peroxidation in a dose-dependent manner.

A vast amount of evidence suggests that aging is associated with a decrease in antioxidant capability and that the age-dependent increase in lipid peroxidation is a consequence of diminished antioxidant protection (Hagihara, Nishigaki, Maseki, & Yagi, 1984). The major antioxidant enzymes, including SOD and CAT, are the first line of the antioxidant defense system against ROS generated *in vivo* during oxidative stress. SOD dismutates superoxide radicals to form hydrogen peroxide, which in turn is decomposed into water and oxygen by CAT, thereby preventing the formation of hydroxyl radicals (Yao et al., 2005). Therefore, these enzymes act cooperatively at different sites in the free radicals' metabolic pathway. MDA and SOD are usually measured at the same time. The activity of SOD reflects the decrease in the body's ability to deal with superoxide anion radicals, while the amount of MDA reflects how badly the cells of the body have been attacked by free radicals.

Many studies have shown that one or more antioxidant enzymes decrease as a consequence of aging (Inal, Kanbak, & Sunal, 2001). In the present study, we found that SOD and CAT activities changed markedly with the EPS-I dose and that these changes had statistical significance when measured in serums. It is likely that the decrease in SOD activity is the main factor in lipid peroxidative damage. EPS-I showed an ability to inhibit lipid peroxidation as shown by the reduction of MDA production in all treated groups. Although EPS-I's antioxidant mechanism is unclear, it is possible that the effects of EPS-I on SOD and CAT are associated with triggering SOD and CAT gene expressions (Pang, Chen, & Zhou, 2000).

4. Conclusion

A novel EPS, called EPS-I, was obtained by ultra-filtration, ion exchange and sizing chromatography from a culture of *L. lactis* subsp. *lactis* 12. EPS-I was mainly composed of fructose and rhamnose with a mean molecular weight of 6.9×10^5 Da. Using *in vitro* antioxidant assays, EPS-I showed strong total antioxidant capacity, as well as an ability to inhibit hydroxyl radical and superoxide anion radical activity. Antioxidant testing *in vivo* showed that EPS-I

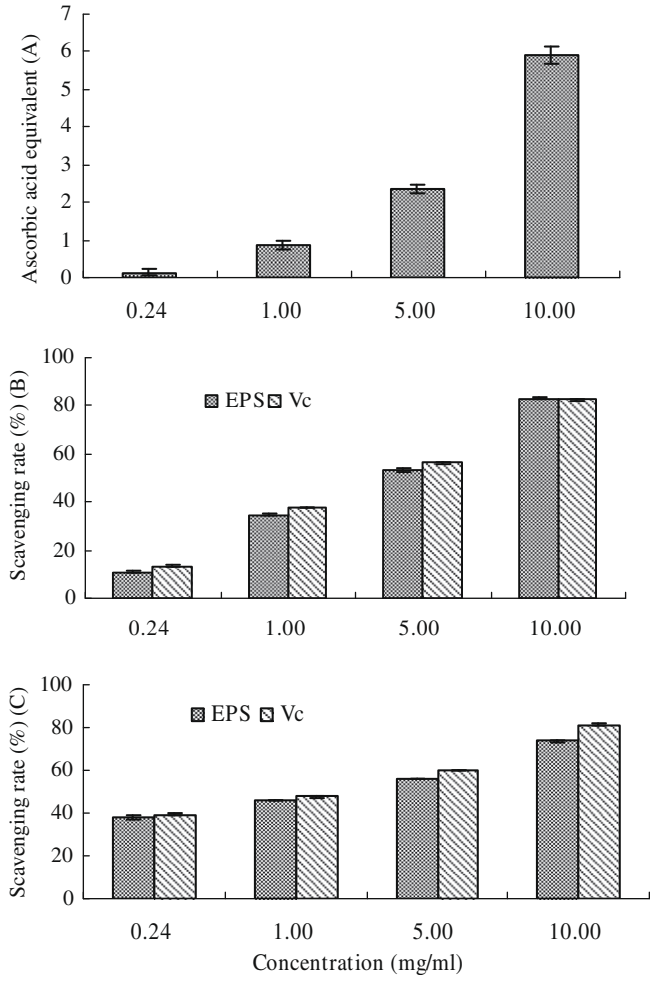

Fig. 3. Total antioxidant capacity (A), EPS-I scavenging activity for superoxide anion (B) and hydroxyl (C) radicals.

Table 1
Effects of EPS-I on CAT and SOD activity, and MDA levels in mice serum.

Groups	Dose (mg/kg)	CAT (U/mL)	SOD (U/mL)	MDA (nmol/mL)
Control		6.36 ± 0.70	91.26 ± 7.13	16.50 ± 2.60
Low dose	40	8.19 ± 1.36[a]	102.30 ± 4.04[b]	12.91 ± 3.21[a]
Middle dose	80	9.58 ± 1.00[b]	108.66 ± 3.27[b]	5.46 ± 1.34[b]
High dose	120	12.73 ± 1.28 [b]	120.04 ± 4.81[b]	2.52 ± 1.26[b]

The data were presented as mean ± SD (n = 8).
[a] Significant difference at the 0.05 level.
[b] Significant difference at the 0.01 level compared with the control group.

administration significantly inhibited MDA formation and increased the activities of antioxidant enzymes including CAT and SOD in mice serums. The results suggest that EPS-I has direct and potent antioxidant capabilities. This study suggests that EPS-I could be used to compensate for declines in total antioxidant capacity and of antioxidant enzymes and non-enzymes, thereby reducing the risk of lipid peroxidation.

Acknowledgements

This work was supported by the State Science and Technology Ministry of the People's Republic of China (Program Nos. 2007AA10Z320, 2007AA10Z357 and 2009GB2C220412) and the K.C. Wong Magna Fund in Ningbo University.

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