Carbohydrate Polymers 98 (2013) 1147–1152

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Exopolysaccharide from *Lactobacillus planterum* LP6: Antioxidation and the effect on oxidative stress

Jing-Yan Li, Man-Man Jin, Jun Meng, Shu-Ming Gao, Rong-Rong Lu *

State Key Laboratory of Food Science and Technology, School of Food Science and Technology, Jiangnan University, 1800 Lihu Avenue, Wuxi, Jiangsu 214122, PR China

ARTICLE INFO

Article history:
Received 26 December 2012
Received in revised form 10 June 2013
Accepted 10 July 2013
Available online 18 July 2013

Keywords:
Exopolysaccharide
Lactic acid bacteria
Isolation and purification
Antioxidant activity
Rat pheochromocytoma line 12

ABSTRACT

An exopolysaccharide (EPS-3) was isolated from the culture of *Lactobacillus planterum* LP6 and purified by ion exchange and gel chromatography. The concentrations required to scavenge 50% of the initial radical for DPPH•, OH• and $O_2^{\bullet -}$ radicals were 1.38, 3.43 and 0.11 mg/mL, respectively. The reducing power ($A_{700\,nm}$) was 0.632 at 5 mg/mL. The cell viability of PC12 was improved by 21.67% at 200 μg/mL of EPS-3. Compared with the H_2O_2 group, the total-antioxidant capacity, activities of superoxide dismutase and catalase were enhanced by 65.81%, 41.34% and 59.05%, respectively. Meanwhile, the level of malondialdehyde and lactate dehydrogenase were decreased by 52.80% and 30.24%. The result indicated that EPS-3 had a notable protective effect against oxidative damage on PC12 cells. The study might lay a theoretical foundation for the comprehensive utilization of lactic acid bacteria source which could result in its application in food systems.

1. Introduction

Reactive oxygen species (ROS), including hydroxyl radical (OH•), superoxide radical ($O_2^{\bullet -}$) and hydrogen peroxide (H_2O_2), are generated as a result of aerobic metabolism during life. Under normal circumstances, ROS plays an important role in life activities. They are normally controlled by the body's antioxidant defense and repair system. However, when they are not removed efficiently due to the diseases and infections and radiations, or they exceed the normal level as a result of the invading of exogenous free radicals or radiation oxidative stress can result (Mates, 2000). Excessive amount of ROS attack cellular components (such as lipids, proteins and nucleic acids), damage the integrity of cells, causing serious of health problem such as cancer, Alzheimer's, Parkinson's and atherosclerosis *etc.* (Chien, Sheu, Huang, & Su, 2007). The application of antioxidants might be an effective therapeutic strategy to alleviate diseases initiated by ROS. However, most synthetic antioxidants are found to have cytotoxicity and cannot be safely used, their applications are limited (Liu, Ooi, & Chang, 1997). For this reason, more attention has been paid to the natural non-toxic antioxidants.

Microbial exopolysaccharides (EPSs) not only have good rheological properties, but also possess some biological activities such as anti-tumor, anti-inflammation, anti-diabetes and macrophage activation (Meng et al., 2010). Lactic acid bacteria (LAB) are identified as Generally Recognized as Safe Foods (GRAS) by the U.S. FDA. As the resident flora of the intestinal track, it is one of the microorganisms that are closely related to human beings. The EPS produced by LAB can contribute to the good texture and taste of fermented products. Besides, owing to its specific glycosidic bond, it cannot be digested by the digestive amylase. That makes it possible to exert the antioxidant activity in organism's gastrointestinal tract, which is the most vulnerable part to oxidation attacks (Choi et al., 2006).

Most studies use either the chemical method *in vitro* to evaluate its free radical scavenging capacity or animal experiments to test its *in vivo* antioxidant activities. However, the results of *in vitro* tests are often not in agreement with each other because of different mechanism of determination. The animal experiments are always time-consuming and can be difficult to reproduce. Cytology is a new research method that has been widely used in the medical and biological research field. It can imitate the cellular microenvironment and it is much relatively easy to visualize and control (Yang et al., 2006), which may help to explore the possible mechanism of biological activity organisms. The PC12 cell is sensitive to oxidative stress, it is often used as a model cell in the antioxidant research of some bioactive substance (Garrido et al., 2011; Kim et al., 2011; Lu et al., 2010; Sun, Shan, Gao, & Tan, 2005; Zhang et al., 2012).

In this paper, a novel EPS was separated and purified from the culture of previously selected LAB strain. Its *in vitro* antioxidant activities and protective effects on oxidative damaged PC12 cells were measured. The study would lay a theoretical foundation for

* Corresponding author. Tel.: +86 0510 85329061; fax: +86 0510 85912155.
E-mail addresses: lurr@jiangnan.edu.cn, lurr2004@hotmail.com (R.-R. Lu).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.07.027

the comprehensive utilization of lactic acid bacteria resource, and expand the application of LAB EPS in food system.

2. Materials and methods

2.1. Materials and reagents

Lactobacillus planterum LP6 was provided by the Research Center of Food Biotechnology, Jiangnan University (Jiangsu, China). The rat pheochromocytoma line 12 (PC12) cells were sourced from Institute of Biochemistry & Cell Biology (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM) was obtained from Gibco BRL, Life Technologies (New York, USA). Newborn Calf Serum (NBS) was purchased from Hangzhou Sijiqing Co. (Hangzhou, China). 2,2-Diphenyl-1-picryhydrazyl (DPPH), 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2-tetrazolium bromide (MTT), DEAE-Sepharose FF® and Sepharose CL-6B® were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals used were purchased from commercial suppliers and of analytical grade.

2.2. Isolation and purification of the EPS

L. planterum LP6 was grown in Man-Rogosa-Sharpe (MRS) broth for 24 h at 37 °C. Cells were removed from culture medium by centrifugation at 3000 × *g* for 10 min at 4 °C. The concentrated supernatant was added to three volumes of 90% (V/V) cold ethanol and held overnight at 4 °C. The precipitate was collected by centrifugation at 3000 × *g* for 10 min and then redissolved in distilled water. The solution was treated with Sevag agent (chloroform: n-butanol = 4:1) and centrifuged to remove free protein. The deproteinized solution was dialyzed (MWCO: 8–14 kDa) against distilled water, concentrated and then lyophilized (Labconco Co., Kansas City, MO, USA) to give the crude EPS (CEPS).

The CEPS (20 mg/mL) solution was subjected to a DEAE-Sepharose FF® anion-exchange chromatography column (2.6 cm × 30 cm) pre-equilibrated with distilled water (pH 7.0), eluted by 0, 0.2, 0.4, 0.6, 0.8 and 1.0 mol/L NaCl at the rate of 1 mL/min. Fractions were collected separately. The total carbohydrate content was determined using the phenol-sulfate method. The main fraction was selected for further separation by the gel-filteration chromatography on a Sepharose CL-6B® column (1.6 cm × 80 cm), eluted with 50 mmol/L NaCl at the rate of 0.5 mL/min. The target EPS was concentrated, dialyzed and lyophilized for further study.

2.3. Determination of free radical scavenging capacities and reducing power

The scavenging effect of EPSs on DPPH free radical was determined according to the method described by Shimada (Shimada, Fujikawa, Yahara, & Nakamura, 1992). The hydroxyl free radical scavenging capacity was assessed using the Fenton system. The scavenging capacity of superoxide anion radical was measured by inhibition of pyrogallol autoxidation (Farhoosh, Golmovahhed, & Khodaparast, 2007). The results were presented as the EC_{50} value, which defined as the concentration required to scavenge 50% of the initial radical.

The reducing power was evaluated by the potassium ferricyanide method described by Yildirim (Yildirim, Mavi, & Kara, 2001).

2.4. Cell culture and treatment

PC12 cells were cultured according to Lu's method (Lu et al., 2010). All experiments were carried out 24 h after cells were plated in 96-well microplate (Corning Incorporated Life Sciences, Lowell, MA) at a density of 1.5×10^5 per well. H_2O_2 was prepared in the medium by several times of dilution before added in. After pretreatment with 200 μg/mL different EPSs for 2 h, H_2O_2 was added to the cultures with the final concentration of 150 μmol/L. The damage was stopped by adding fresh medium. The control group cells were only treated with fresh medium containing no H_2O_2 and EPS.

2.5. Assay of cell viability

The cell viability was measured in 96-well plates by MTT method as described by Sun (Sun et al., 2005). The MTT was added to the medium at a final concentration of 0.5 mg/mL. After 4 h incubation, the supernatant was removed and 150 μL dimethyl sulfoxide was added. The mixture was shaken up until formazan crystals were dissolved. The absorbance at 570 nm was measured with a MK3® microplate reader (Thermo Fisher Scientific Inc, Waltham, MA, USA). Cell viability was expressed as mean percentage of viable cells compared with a control group.

2.6. Morphological assay

To analyze the effect of EPS-3 on cell morphology, cells were cultured at a density of 1.5×10^5 per well in 96-well microplate for 24 h. After different pretreatment, cells were photographed by phase contrast microscopy (Olympus Corporation, Tokyo, Japan).

2.7. T-AOC, CAT, SOD, LDH and MDA assays

The PC12 cells were washed after treatment as above, and then scraped into ice-cold PBS. The cell suspension was treated by repeated freeze-thaw rock twice to break the cell. Then it was centrifuged at 1000 × *g* for 5 min at 4 °C. The activities of total-antioxidant capacity (T-AOC), catalase (CAT) and superoxide dismutase (Larocque, Brisson, Thérisod, Perry, & Caroff, 2003) were determined using Assay Kits (Institute of Biological Engineering of Nanjing Jiancheng, Nanjing, China) according to the protocols, as well as the leakage of lactate dehydrogenase (LDH) and the content of malondialdehyde (MDA).

2.8. Statistical analysis

All the experiments were conducted at least in triplicate. Significant analysis was analyzed by one-way ANOVA, using SPSS 16.0 for windows® software (SPSS Inc., Chicago, IL, USA). Results were presented as means ± standard errors.

3. Results and discussion

3.1. Isolation and purification of the EPS

The anion exchange chromatographic elution profile of CEPS on a DEAE-Sepharose FF® column was shown in Fig. 1A. Three polysaccharide fractions were obtained and collected respectively. They were the neutral component EPS-1 and EPS-2 that eluted by deionized water, and the acidic component EPS-3 eluted by 0.4 mol/L NaCl. The content of carbohydrate was detected by using the phenol-sulfuric acid assay. As a result, EPS-3 was the major part of CEPS, possessing 71.3% of the total amount.

Then EPS-3 was re-fractionated over a size-exclusion chromatography Sepharose CL-6B® column. As shown in Fig. 1B, there was a single and sharp symmetrical peak in its elution profile, indicating that EPS-3 was homogeneous.

Table 1
Free radical scavenging capacities and reducing power of different EPSs.

	DPPH radical[a]	Superoxide radical[a]	Hydroxyl radical[a]	Reducing power[b]
CEPS	1.62 ± 0.07^{B}	4.06 ± 0.09^{C}	0.17 ± 0.01^{B}	0.882 ± 0.029^{C}
EPS-1	4.46 ± 0.09^{C}	>8.00	0.34 ± 0.04^{C}	0.521 ± 0.017^{A}
EPS-2	>5.00	>8.00	0.87 ± 0.03^{D}	0.496 ± 0.019^{A}
EPS-3	1.38 ± 0.05^{B}	3.43 ± 0.09^{B}	0.11 ± 0.01^{A}	0.632 ± 0.021^{B}
Vc	0.32 ± 0.01^{A}	2.48 ± 0.08^{A}	0.15 ± 0.01^{B}	1.492 ± 0.064^{D}

Data are expressed as means ± SD of triplicates. Different superscript characters (A, B, C, D) indicate the significant difference at $p < 0.05$ level within a same column.
[a] EC_{50}, mg/mL.
[b] Absorbance at 700 nm tested at concentration of 5 mg/mL.

3.2. Free radical scavenging capacities and reducing power of EPSs

Since the antioxidant mechanisms for *in vitro* assay methods were diverse, the antioxidant activity should be determined by different assays (Moure, Dominguez, & Parajo, 2006). In this study, four indexes including the scavenging capacities of DPPH•, OH• and $O_2^{\bullet -}$ were measured, as well as the reducing power, using ascorbic acid (Vitamin C, Vc) as the reference. As shown in Table 1, the EC_{50} values of scavenging rate of EPS-3 for three radicals were 1.38, 3.43 and 0.11 mg/mL, respectively. The reducing power was 0.632 at 700 nm. They were significantly higher than those of CEPS and the other two neutral fractions ($p < 0.05$). However, it was still not as good as Vc ($p < 0.05$). These results demonstrated that EPS-3 had a relatively notable effect on scavenging of free radicals and obvious reducing power. EPS-3 was not only the main composition of CEPS, but also the chief antioxidative component.

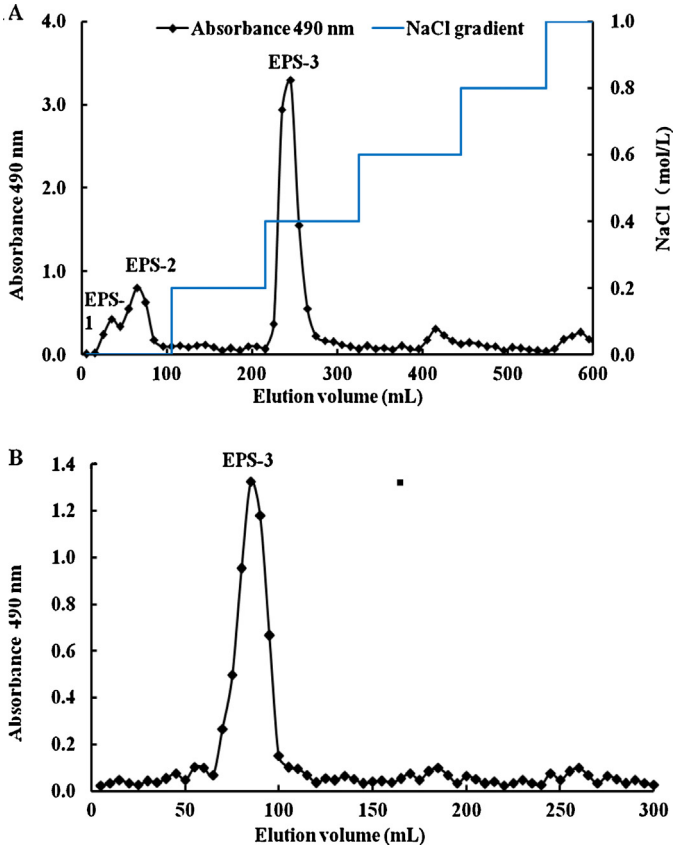

Fig. 1. Purification of CEPS produced by *L. planterum* LP6. Stepwise elution curve of CEPS on DEAE-Sepharose FF® column with 0–1.0 mol/L NaCl at a flow rate of 1 mL/min (A). The main fraction EPS-3 was applied onto a Sepharose CL-6B® column with 50 mmol/L NaCl at the rate of 0.5 mL/min (B).

The result was similar with Liu's (Liu et al., 2009) and Xu's (Xu, Shang, & Li, 2011) reports. These antioxidative EPSs contained higher content of uronic acid than usual, which made the molecules carry more negative charge. The higher charge increased the intramolecular repulsive force and made the molecules more extended. This reduced the steric hindrance for radical to attack (Duh, Tu, & Yen, 1999; Kishk & Al-Sayed, 2007). Thus, the free radical became more likely to be scavenged, and the EPSs exerted good antioxidant activity.

3.3. Effects of different EPSs on viability of H_2O_2 treated PC12 cell

As shown in Fig. 2, PC12 cells were extremely sensitive to H_2O_2 injury. It exhibited marked decrease in the cell viability after exposure to 150 μmol/L H_2O_2. The viability almost dropped to half compared with the control group. However, when cells were pretreated with 200 μg/mL different EPSs for 2 h before exposure to H_2O_2, the cell toxicity was attenuated significantly. Obviously increasing of the cell viability rate indicated that each EPS had a protective effect on cellular oxidative damage ($p < 0.05$). EPS-3 and CEPS expressed much stronger cytoprotective action than the other two EPSs, especially EPS-3, which raised the survival rate by 21.67% ($p < 0.01$). This result was in accordance with the *in vitro* antioxidant performance.

3.4. Effects of EPS-3 on H_2O_2-induced morphological alterations PC12 cells

Fig. 3 shows the morphology of PC12 cells damaged by H_2O_2 and protected by EPS-3. Fig. 3A demonstrates the common state of cells under normal cultivation. Cells grew well, showing long fibrous structures with clear boundaries. Fig. 3B was the cell injured by 150 μmol/L H_2O_2 for 24 h. There were significant changes in the cell form. Cells no longer stretched pseudopodia and shrank into round shaped. Some of them were indicated to congregate and even

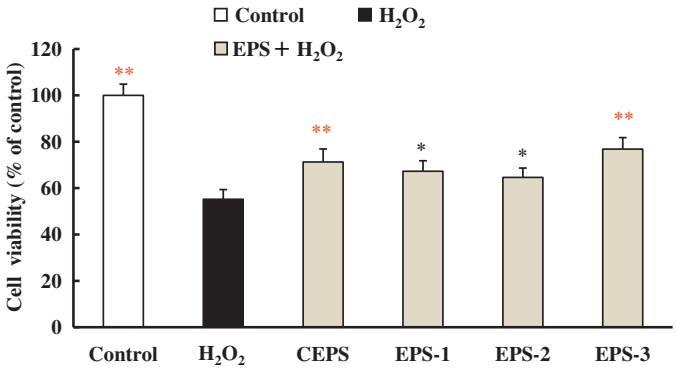

Fig. 2. EPS inhibits the reduction of cell viability induced by H_2O_2 in PC12 cells. PC12 cells were untreated (Control), incubated with H_2O_2 (150 μmol/L) for 24 h (H_2O_2-group), and pretreated with different kinds of EPS (200 μg/mL) for 2 h before exposed to H_2O_2 (150 μmol/L) for 24 h. *$p < 0.05$, **$p < 0.01$ *vs.* H_2O_2-group.

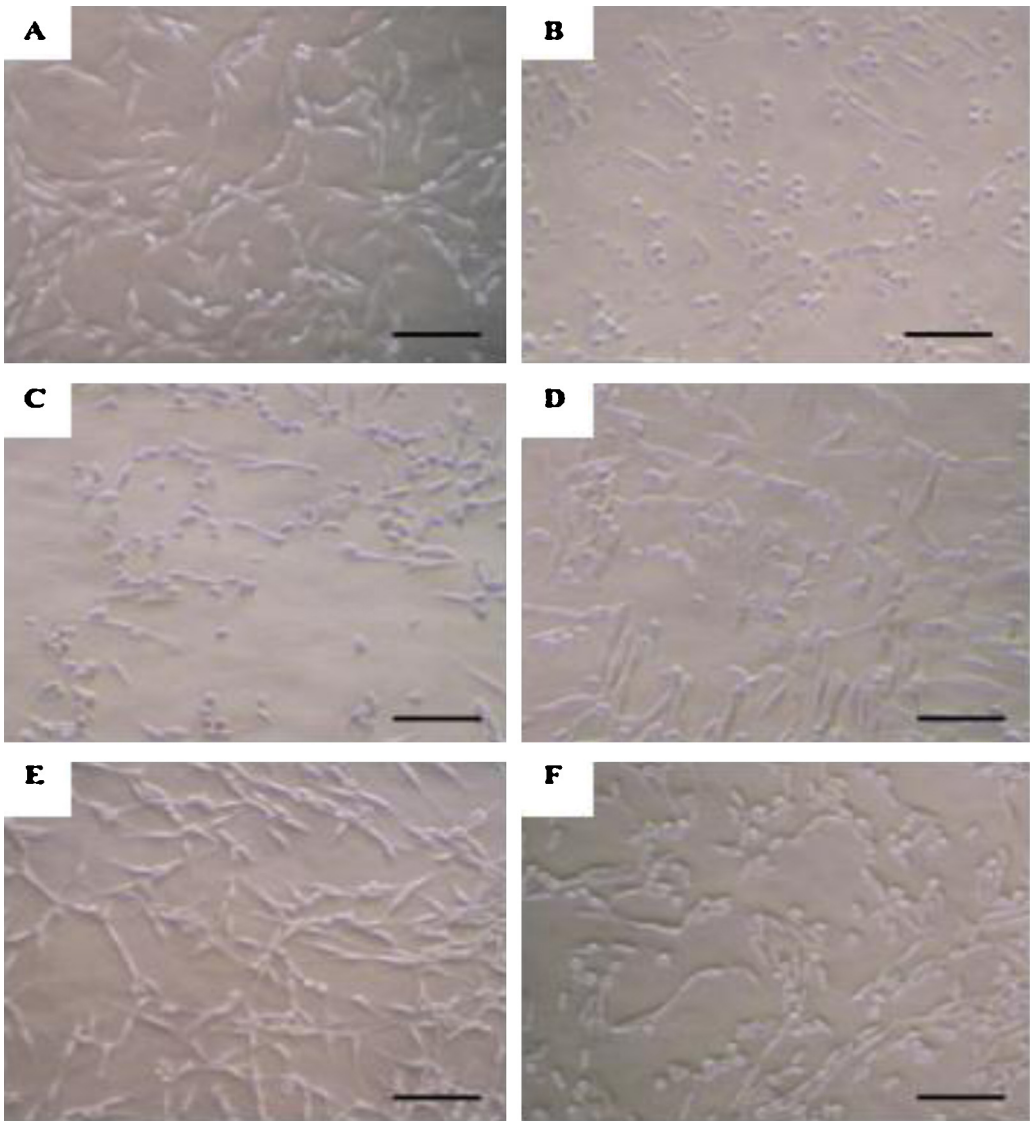

Fig. 3. Effects of EPS-3 on morphological alterations of H_2O_2-damaged PC12 cells. PC12 cells were untreated (A), incubated with H_2O_2 (150 μmol/L) for 24 h (B), pretreated with various concentrations of EPS-3 (100, 400 μg/mL) for 2 h before exposed to H_2O_2 (150 μmol/L) for 24 h (C and D), and treated with EPS-3 (100, 400 μg/mL) alone for 24 h (E and F). After different treatments, cells were photographed under phase contrast microscopy. *Bar* 30 μm.

lyzed to yield debris after exposure to H_2O_2. However, when the cells were pre-incubated with EPS-3 (100 and 400 μg/mL), H_2O_2 induced that the cell toxicity was attenuated significantly (Fig. 3C and D), and exhibited the similar state compared with control group. These results illustrated that EPS-3 did significantly protection on PC12 cells from H_2O_2-induced cytotoxicity. Cells incubated with 100 μg/mL EPS-3 (Fig. 3E) grew better than cells of control group, indicating that EPS-3 had a growth promoting effect on normal cells. Cells incubated with 400 μg/mL EPS-3 (Fig. 3F) tended to be round shaped that was similar with those damaged by H_2O_2. It demonstrated that EPS-3 might kill the cell at high concentrations. The result was similar to Shen's report that EPS of high concentration might be harmful to cells (Shen, Song, Wu & Zhang, 2011).

3.5. Effects of EPS-3 on viability of H_2O_2 treated PC12 cell

As shown in Fig. 4, EPS-3 protected the cell in a dose dependent manner ($p < 0.05$), it increased with the increasing concentration. Moreover, it could be seen that EPS-3 has both a promoting effect at low concentrations and a suppressing effect at high concentration on cell growth. It was supposed that at low concentration, EPS-3 could inhibit the generation of MDA, reduce the cross-linking of membrane and restrain the decline in membrane fluidity and signal transmission. However, the suppressing effect at high concentration may come from its antitumor activity that inducing tumor cell apoptosis in some way else (Shen et al., 2011).

H_2O_2 could get through the cell membrane easily, generate vast of high cytotoxic free radicals *via* intracellular metabolism. The notable radical scavenging capacity and reducing power of EPS endowed it with antioxidant protective activity to some extent. The results showed that it could prevent membrane lipid peroxidation, maintained the integrity of the cell, and improved its viability.

3.6. Effects of EPSs on T-AOC, T-SOD, CAT, LDH and MDA

Enzymatic defense of cells against ROS involved the cooperative action of several enzymes, including the two core antioxidant enzymes SOD and CAT. SOD catalyses the dismutation of superoxide radical to H_2O_2, while CAT is responsible for scavenging it (Chance, Sies, & Boveris, 1979). Treatment with 150 μmol/L H_2O_2 caused a significant ($p < 0.05$) decrease in activities of T-AOC, SOD and CAT (Table 2). However, the preincubation of PC12 cells with

Table 2
Effects of EPS-3 on T-AOC, SOD, CAT, MDA and LDH in H_2O_2-induced PC12 cells.

	T-AOC (U/mg)	SOD (U/mg)	CAT (U/mg)	MDA (nmol/mg)	LDH (U/L)
Control	9.39 ± 0.29^{E}	201.22 ± 3.99^{D}	20.66 ± 1.11^{D}	2.32 ± 0.20^{A}	557.42 ± 19.01^{A}
H_2O_2	2.10 ± 0.14^{A}	96.9 ± 2.98^{A}	5.69 ± 0.99^{A}	7.19 ± 0.52^{D}	1126.32 ± 21.06^{C}
CEPS	6.06 ± 0.24^{C}	123.48 ± 2.21^{B}	14.35 ± 0.52^{C}	3.86 ± 0.18^{B}	829.75 ± 23.13^{B}
EPS-1	4.09 ± 0.33^{B}	129.72 ± 3.02^{B}	7.46 ± 0.73^{B}	4.72 ± 0.28^{C}	854.33 ± 21.21^{B}
EPS-2	4.99 ± 0.17^{B}	132.90 ± 1.66^{B}	9.29 ± 0.85^{B}	5.21 ± 0.11^{C}	988.99 ± 22.33^{C}
EPS-3	8.28 ± 0.26^{D}	180.08 ± 1.87^{C}	17.89 ± 0.33^{C}	3.39 ± 0.15^{B}	785.63 ± 17.75^{B}

Data are expressed as means ± SD of triplicates. Different superscript characters (A, B, C, D) indicate the significant difference at $p < 0.05$ level within a same column.

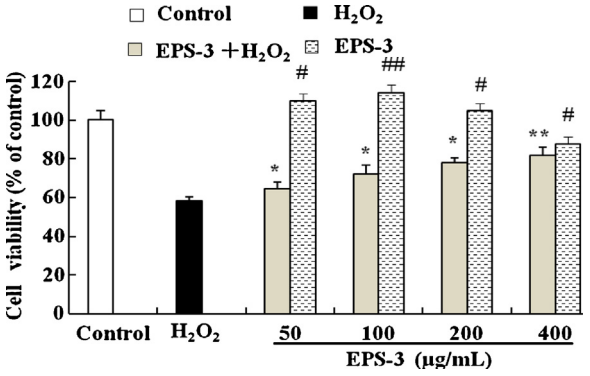

Fig. 4. EPS-3 inhibited the reduction of cell viability induced by H_2O_2 in PC12 cells. PC12 cells were untreated (Control), incubated with H_2O_2 (150 μmol/L) for 24 h (H_2O_2), pretreated with various concentrations of EPS-3 (50, 100, 200 and 400 μg/mL) for 2 h before exposed to H_2O_2 (150 μmol/L) for 24 h (EPS-3 + H_2O_2), and treated with EPS-3 alone for 24 h (EPS-3). $^{*}p < 0.05$, $^{**}p < 0.01$ *vs.* H_2O_2, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ *vs.* control.

200 μg/mL EPSs clearly inhibited the active damping. EPS-3 performed best ($p < 0.05$) enhancing each activity by 65.81%, 41.34% and 59.05% respectively compared with the H_2O_2 group. This was consistent with the results of the *in vitro* test.

MDA was intermediate product of lipid peroxidation. It indicates the degree of oxidative damage. As shown in Table 2, PC12 cells protected by EPS caused a great decrease by 52.80% in the intracellular MDA level compared with the H_2O_2 group ($p < 0.05$), indicating that EPS inhibited the oxidation in cells significantly. The release of LDH was another indicator of cellular damage and cytotoxicity (Bagchi, Bagchi, Hassoun, & Stohs, 1995). As shown in Table 2, the release of LDH in EPS treated group was reduced by 30.24% showing that the cells were protected.

Almost all the polysaccharide reported could enhance the activities of the antioxidant enzyme system. The superior antioxidant protective effect of EPS-3 might well benefit from their enzyme activity enhancements. It inhibited the lipid peroxidation, helped to maintain the integrity of cell and prevented the invasion of external ROS.

4. Conclusion

In this study, we isolated and purified a novel exopolysaccharide EPS-3 from the fermentation metabolites of *L. planterum* LP6. It enhanced the activities of the intracellular antioxidant enzyme system, inhibited lipid peroxidation and helped to maintain the cell integrity. The development of the antioxidant EPS from LAB fermentation may open up a comprehensive utilization of lactic acid bacteria resource and extend to applications in food systems.

Conflict of interest

The authors have declared that there is no conflict of interest.

Acknowledgement

This study was supported by the grants from the State Natural Sciences Foundation of China (No 31071491), "the Twelfths 5-year" National Key Technology R&D Program of China (2012BAD33B05) and the 863 project (2013AA102207).

References

Bagchi, D., Bagchi, M., Hassoun, E., & Stohs, S. (1995). In vitro and in vivo generation of reactive oxygen species, DNA damage and lactate dehydrogenase leakage by selected pesticides. *Toxicology*, *104*(1), 129–140.
Chance, B., Sies, H., & Boveris, A. (1979). Hydroperoxide metabolism in mammalian organs. *Physiological Reviews*, *59*(3), 527–605.
Chien, P. J., Sheu, F., Huang, W. T., & Su, M. S. (2007). Effect of molecular weight of chitosans on their antioxidative activities in apple juice. *Food Chemistry*, *102*(4), 1192–1198.
Choi, S. S., Kim, Y., Han, K. S., You, S., Oh, S., & Kim, S. H. (2006). Effects of Lactobacillus strains on cancer cell proliferation and oxidative stress in vitro. *Letters in Applied Microbiology*, *42*(5), 452–458.
Duh, P. D., Tu, Y. Y., & Yen, G. C. (1999). Antioxidant Activity of Water Extract of Harng Jyur *Chrysanthemum morifolium* Ramat. *LWT-Food Science and Technology*, *32*(5), 269–277.
Farhoosh, R., Golmovahhed, G. A., & Khodaparast, M. H. H. (2007). Antioxidant activity of various extracts of old tea leaves and black tea wastes (*Camellia sinensis* L.). *Food Chemistry*, *100*(1), 231–236.
Garrido, J., Gaspar, A., Manuela Garrido, E., Miri, R., Tavakkoli, M., Pourali, S., et al. (2011). Alkyl esters of hydroxycinnamic acids with improved antioxidant activity and lipophilicity protect PC12 cells against oxidative stress. *Biochimie*, *94*(4), 916–917.
Kim, H. G., Kim, G. W., Oh, H., Yoo, S. Y., Kim, Y. O., & Oh, M. S. (2011). Influence of roasting on the antioxidant activity of small black soybean (Glycine max L. Merrill). *LWT-Food Science and Technology*, *44*(4), 992–998.
Kishk, Y. F. M., & Al-Sayed, H. (2007). Free-radical scavenging and antioxidative activities of some polysaccharides in emulsions. *LWT-Food Science and Technology*, *40*(2), 270–277.
Larocque, S., Brisson, J. R., Thérisod, H., Perry, M. B., & Caroff, M. (2003). Structural characterization of the O-chain polysaccharide isolated from Bordetella avium ATCC 5086: Variation on a theme. *FEBS Letters*, *535*(1), 11–16.
Liu, F., Ooi, V. E. C., & Chang, S. T. (1997). Free radical scavenging activities of mushroom polysaccharide extracts. *Life Sciences*, *60*(10), 763–771.
Liu, J., Luo, J., Ye, H., Sun, Y., Lu, Z., & Zeng, X. (2009). Production, characterization and antioxidant activities in vitro of exopolysaccharides from endophytic bacterium Paenibacillus polymyxa EJS-3. *Carbohydrate Polymers*, *78*(2), 275–281.
Lu, R.-R., Qian, P., Sun, Z., Zhou, X.-H., Chen, T.-P., He, J.-F., et al. (2010). Hempseed protein derived antioxidative peptides: Purification, identification and protection from hydrogen peroxide-induced apoptosis in PC12 cells. *Food Chemistry*, *123*(4), 1210–1218.
Mates, J. (2000). Effects of antioxidant enzymes in the molecular control of reactive oxygen species toxicology. *Toxicology*, *153*(1), 83–104.
Meng, F., Liu, X., Jia, L., Song, Z., Deng, P., & Fan, K. (2010). Optimization for the production of exopolysaccharides from Morchella esculenta SO-02 in submerged culture and its antioxidant activities in vitro. *Carbohydrate Polymers*, *79*(3), 700–704.
Moure, A., Dominguez, H., & Parajo, J. C. (2006). Antioxidant properties of ultrafiltration-recovered soy protein fractions from industrial effluents and their hydrolysates. *Process Biochemistry*, *41*(2), 447–456.
Shen, W., Song, D., Wu, J., & Zhang, W. (2011). Protective effect of a polysaccharide isolated from a cultivated Cordyceps mycelia on hydrogen peroxide-induced oxidative damage in PC12 cells. *Phytotherapy Research*, *25*(5), 675–680.
Shimada, K., Fujikawa, K., Yahara, K., & Nakamura, T. (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry*, *40*(6), 945–948.
Sun, C., Shan, C., Gao, X., & Tan, R. (2005). Protection of PC12 cells from hydrogen peroxide-induced injury by EPS2, an exopolysaccharide from a marine filamentous fungus Keissleriellasp. YS4108. *Journal of Biotechnology*, *115*(2), 137–144.

Xu, R., Shang, N., & Li, P. (2011). In vitro and in vivo antioxidant activity of exopolysaccharide fractions from Bifidobacterium animalis RH. *Anaerobe*, *17*(5), 226–231.

Yang, B., Wang, J., Zhao, M., Liu, Y., Wang, W., & Jiang, Y. (2006). Identification of polysaccharides from pericarp tissues of litchi (Litchi chinensis Sonn.) fruit in relation to their antioxidant activities. *Carbohydrate Research*, *341*(5), 634–638.

Yildirim, A., Mavi, A., & Kara, A. A. (2001). Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. *Journal of Agricultural and Food Chemistry*, *49*(8), 4083–4089.

Zhang, Q.-X., Ling, Y.-F., Sun, Z., Zhang, L., Yu, H.-X., Kamau, S. M., et al. (2012). Protective effect of whey protein hydrolysates against hydrogen peroxide-induced oxidative stress on PC12 cells. *Biotechnology Letters*, *34*(11), 2001–2006.