Carbohydrate Polymers 81 (2010) 607–611

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Preparation and biological activities of an exopolysaccharide produced by *Enterobacter cloacae* Z0206

Mingliang Jin[a], Youming Wang[a], Chunlan Xu[a,b], Zeqing Lu[a], Ming Huang[a], Yizhen Wang[a,*]

[a] *The Key Laboratory of Molecular Animal Nutrition of Ministry of Education, College of Animal Sciences, Zhejiang University, No. 164 Qiutao North Road, Hangzhou 310029, PR China*
[b] *College of Life Sciences, Northwestern Polytechnical University, Xi'an 710072, PR China*

ARTICLE INFO

Article history:
Received 28 December 2009
Received in revised form 7 March 2010
Accepted 9 March 2010
Available online 16 March 2010

Keywords:
Exopolysaccharide
Enterobacter cloacae
Immunomodulation
Antioxidant

ABSTRACT

A water-soluble extracellular polysaccharide (EPS-1) was isolated from the submerged culture broth of *Enterobacter cloacae* Z0206 through fermentation, ethanol precipitation, anion-exchange and gel-permeation chromatography. Its structural analysis showed that the average molecular weight of EPS-1 was 23,928 Da, and it was composed of glucose, mannose and galactose with a molar ratio of 6.860:1.180:0.455. It was hypothesized that EPS-1 belongs to the α-type heteropolysaccharide with pyran group. Bioactivity tests *in vivo* showed that the administration of EPS-1 to cyclophosphamide (CP)-exposed animals resulted in significant increase and recovery of B lymphocyte proliferation, tumor necrosis factor α (TNF-α) production and activities of antioxidant enzymes (superoxide dismutase (SOD) and glutathione peroxidase (GPx)). It is suggested that EPS-1 could provide protection against CP-induced immunosuppression and oxidative damage in mice model, and it may act as a potent immunomodulatory and antioxidant agent.

1. Introduction

In recent years, many polysaccharides have been isolated from mushrooms, fungi, yeasts, algae, lichens and plants (Liu et al., 2007). They have emerged as an important class of bioactive natural products in the biochemical and medical areas due to their specific biological activities such as immunostimulating (Xu, Wang, Jin, & Yang, 2009), antioxidant (Luo & Fang, 2008), antiviral (Wang, Ooi, & Ang, 2007), antitumor effects (Tong, Xia, Feng, Sun, & Gao, 2009). In many Asian countries, several immunoceuticals composed of polysaccharides have been accepted such as lentinan, krestin and schizophyllan (Zhang, Cui, Cheung, & Wang, 2007).

Enterobacter cloacae Z0206, a bacterial strain, can produce large amounts of exopolysaccharides. It has been reported that glycoproteins from *E. cloacae* showed antitumor effects on mice with S180 tumors (real), and F3, one of the glycoprotein components, could distinctly inhibit QGY7703 (liver cancer), A549 (glandular cancer of the lungs), Kato III (gastric carcinoma) and Sw1116 (intestinal cancer) cell strains (Zhang, Ren, & Chen, 2002). In our previous studies (Xu et al., 2009), we found that *E. cloacae* Z0206 could accumulate selenium (Se) in the form of Se-enriched exopolysaccharide efficiently during cultivation with selenium. Administration of this Se-enriched exopolysaccharide to cyclophosphamide (CP)-exposed mice resulted in improvement of cellular and humoral immune responses.

However, little is known about the exact pharmacological effects and structure–function relationship of exopolysaccharide without Se derived from *E. cloacae*, which would allow a better understanding of the functional effects about this macromolecule. The objective of the current study was to extract and purify the major exopolysaccharide produced by *E. cloacae* Z0206 using DEAE-52 cellulose and Sephadex G-100 column chromatography. Based on the experiments above, the possible effects of this exopolysaccharide on the immune functions and antioxidant status of immunocompromised hosts were further investigated by establishment of CP-induced immunosuppressive mice.

2. Materials and methods

2.1. Materials

The exopolysaccharide-producing bacterial strain identified as *E. cloacae* Z0206 was identified and kept in our laboratory, and it has been collected by China General Microbiological Culture Collection Center (CGMCC). CP, lipopolysaccharide (LPS), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), dimethyl sulfoxide (DMSO) and RPMI-1640 medium were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The

* Corresponding author. Tel.: +86 571 86951729; fax: +86 571 86994963.
E-mail address: yzwang@zju.edu.cn (Y. Wang).

0144-8617/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2010.03.020

sheep red blood cells (SRBCs), used as an antigenic material, were delivered by the Institute of Feed Science, Zhejiang University, kept in Alsever's medium, and washed three times with PBS before use.

2.2. Microorganism cultivation

E. cloacae Z0206 was initially grown on PDA medium (fresh potato, 20% (w/v); dextrose, 2.0%; agar, 2.0%) at 30 °C for 1 day, and then transferred to 250 mL flasks containing 80 mL of seed culture medium (fresh potato, 20% (w/v); dextrose, 2.0%; peptone, 0.2%; yeast extract, 0.3%) and incubated on a rotary shaker at 250 rpm for 18 h at 30 °C.

Cultivation medium contained: dextrose, 2.5%; peptone, 0.5%; yeast extract, 0.5%, K_2HPO_4, 0.2%; KH_2PO_4, 0.1% and $MgSO_4{\cdot}7H_2O$, 0.05%. The exopolysaccharides production was performed in a 10 dm^3 bioreactor (Shanghai Biotech Ltd., China) with 7 dm^3 cultivation medium at 30 °C for 2 days. The initial pH was 7.5 and the inoculation volume was 5.0% (v/v). Aeration rate (1.0 vvm), growth temperature, foam level, pH and dissolved oxygen tension were controlled and/or measured by the bioreactor control unit.

2.3. Preparation of crude exopolysaccharides

After cultivation, the broth was centrifuged at 4500 × *g* for 20 min to remove the mycelia. The supernatant was evaporated under reduced pressure at 50 °C, then precipitated upon addition of 4 volumes of cold 95% EtOH and kept at −20 °C overnight. The resulting precipitate was collected by centrifugation at 7600 × *g* for 15 min at 4 °C. The precipitates were dissolved in distilled water and deproteinized by a combination of papain and trypsin enzymolysis and the Sevag method (Staub, 1965), then precipitated with 4 volumes of cold 95% EtOH at −20 °C overnight. After centrifugation at 7600 × *g* for 15 min at 4 °C, successive washes with anhydrous ethanol, acetone and ether, and drying under vacuum at 40 °C, the crude exopolysaccharides were obtained.

2.4. Purification of exopolysaccharides

The crude exopolysaccharides were redissolved in distilled water and subsequently applied to a DEAE-52 anion-exchange chromatography column (2.6 cm × 50 cm), eluting successively with distilled water and a linear gradient of 0–0.2 M NaCl with a flow rate of 0.5 mL/min. The yielded fractions were combined according to the total carbohydrate content quantified by the phenol-sulfuric acid method (Dubois, Gilles, Harmilton, Rebers, & Smith, 1956). The main peak obtained was concentrated and further fractionated on a Sephadex G-100 column (1.6 cm × 50 cm) eluted with 0.05 M NaCl at a flow rate of 0.4 mL/min in the same manner to yield completely separated fractions. The main fraction was collected, dialyzed and lyophilized to obtain a white purified exopolysaccharide, which was subjected to subsequent analyses.

2.5. Assay for molecular weight

The average molecular weight of the polysaccharide was measured by gel-permeation chromatography (GPC) with an Ultrahydrogel Linear Column (7.8 mm × 300 mm) on a Waters high performance liquid chromatography (HPLC, Waters-2690, Massachusetts, USA). The column was operated at 34 °C and eluted with 0.1 M phosphate buffer (pH 7.0) at a flow rate of 0.8 mL/min. The eluent was monitored with a refractive index detector (RID, Waters-2410, USA). The column was calibrated by various standard dextrans. All data provided by the GPC system were collected and analyzed using the Millennium software package.

2.6. Monosaccharide components of polysaccharide

The polysaccharide (50 mg) was hydrolyzed into monosaccharides with 2 mL of 1 M H_2SO_4 at 100 °C for 10 h, and neutralized with $BaCO_3$ to pH 7.0. After filtration through a 0.45 μm membrane, the mixture was analyzed by HPLC (Waters-2690, USA) equipped with RID and a Sugar-pak-1 column (Waters, USA). Ultrapure water was used as the solvent at a flow rate of 0.7 mL/min and 25 μL of sample or glucose, mannose and galactose each as a standard were injected.

2.7. Infrared spectrophotometer (IR)

The IR spectrum of the polysaccharide was determined using a Fourier transform infrared (FTIR) spectrophotometer (Nicolet Nexus 670, Thermo Electron, USA) equipped for detecting functional groups. The purified polysaccharide was ground with KBr powder and pressed into pellets for FTIR measurement in the frequency range of 4000–400 cm^{-1}.

2.8. Animals and treatment

40 ICR (Institute of Cancer Research) male mice (18 ± 2 g) were randomly divided into four groups of ten each. Two immunosuppressed groups (CP and CP + EPS-1) were administered with EPS-1 (0 and 400 mg/kg body weight (B.W.)) by gavage once daily for 14 days, and CP was given intraperitoneally at 50 mg/kg B.W. on the 12th day. Control mice received the same volume of 0.9% normal saline. The EPS-1 group was given EPS-1 at 400 mg/kg B.W. by gavage once daily for 14 days. Animals of all groups were challenged with 0.2 mL of 0.1% SRBCs (i.p.) on the 10th day. The animals were sacrificed by cervical dislocation 12 h after the last dose. Spleen, liver and blood samples were collected for further analysis.

2.9. Splenic lymphocyte proliferation assay

Single-cell suspensions of splenic lymphocytes were prepared (Kaspers, Lillehoj, Jenkins, & Pharr, 1994) and placed into 96-well flat-bottomed microplates at a concentration of 5×10^6 cells/mL with RPMI-1640 media, adding 100 μL of LPS (10 μg/mL) or complete medium (controls). After incubation for 44 h at 37 °C in a humidified 5% CO_2 atmosphere, 20 μL MTT (5 mg/mL) was added into each well, followed by incubation for another 4 h, and then 100 μL of DMSO was added into each well to dissolve the precipitation completely. The light absorbance was measured at 570 nm with Enzyme-linked Immunosorbent Assay Reader (Model BIO-RAD-550, USA).

2.10. Cytokine determination in serum

Blood was obtained from the heart of treated and control animals and allowed to stand for 1 h at 37 °C. After centrifugation at 6000 × *g* for 10 min at 4 °C, the blood serum was collected and kept frozen until use. The presence of TNF-α in blood serum was determined using the mouse ELISA Set (BD OptEIA, BD Biosciences PharMingen, San Diego, CA, USA).

2.11. Antioxidant status assay in liver

Livers of mice were excised, cleaned and homogenized in 1:5 volumes of normal saline solution. The homogenate was centrifuged at 1700 × *g* for 10 min at 4 °C. The supernatant fractions were collected and activities of SOD and GPx were determined by spectrophotometric methods using corresponding diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.12. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA), followed by Dunnett's *t*-test. Results were presented as means ± S.D. Differences between groups were considered statistically significant at the 5% ($p < 0.05$) level.

3. Results

3.1. Isolation and purification of exopolysaccharides

Crude exopolysaccharides were precipitated with ethanol from the centrifuged and deproteinated culture broth of *E. cloacae* Z0206. The precipitate was fractionated on a DEAE-52 cellulose column with distilled water and a gradient elution of NaCl in the range of 0–0.2 M, leading to the isolation of two polysaccharide sub-fractions, NPS and APS (Fig. 1). Sub-fraction NPS was a neutral polysaccharide as it was eluted with water, and sub-fraction APS should be acidic as it was eluted with NaCl solution. NPS was the major component, shown by its peak areas in anion-exchange chromatography. For convenient preparation and high content, NPS fractions were collected, and further fractionated on a Sephadex G-100 column eluted with 0.05 M NaCl, resulting in one sub-fraction, EPS-1 (Fig. 2).

The GPC profile showed that EPS-1 was a homogeneous polysaccharide with an average molecular weight of 23,928 Da. The

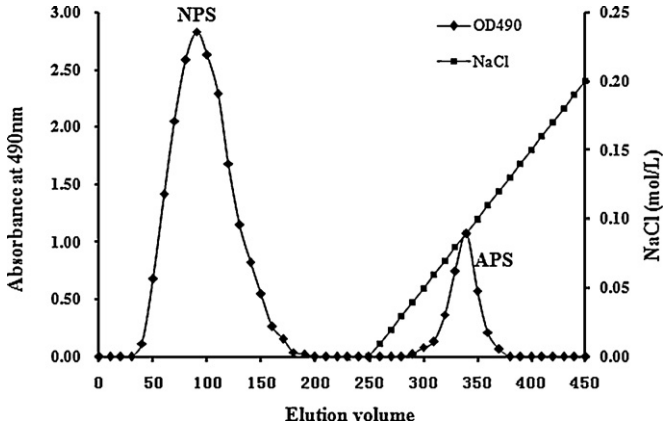

Fig. 1. Anion-exchange chromatography of crude exopolysaccharides produced by *Enterobacter cloacae* Z0206. Column: DEAE-52 cellulose (2.6 cm × 50 cm); flow rate: 0.5 mL/min; fraction volume: 5 mL. NPS: neutral polysaccharide (eluted with distilled water); APS: acidic polysaccharide (eluted with a linear gradient of 0–0.2 M NaCl).

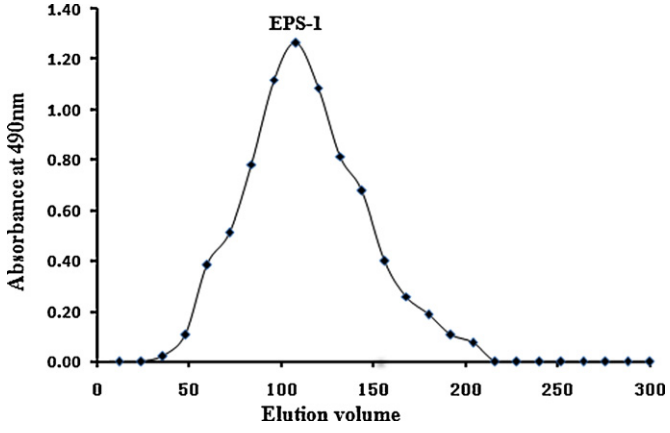

Fig. 2. Gel-permeation chromatography of NPS eluting with 0.05 M NaCl. Column: Sephadex G-100 (1.6 cm × 50 cm); flow rate: 0.4 mL/min; fraction volume: 5 mL.

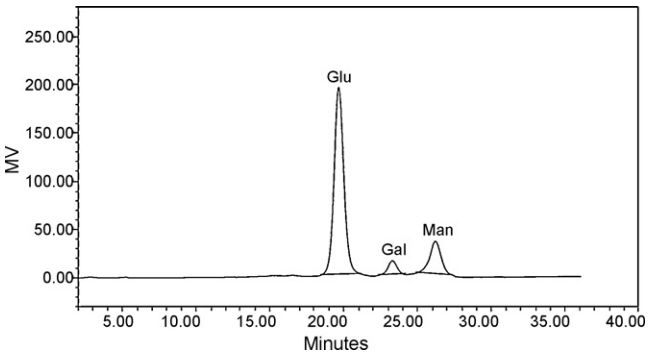

Fig. 3. HPLC analysis of monosaccharide components of EPS-1.

monosaccharide composition of EPS-1 was determined by the sulfuric acid hydrolysis and HPLC analysis methods (Fig. 3). The results indicated that EPS-1 was composed of glucose, mannose and galactose with a molar ratio of 6.860:1.180:0.455.

3.2. IR

The FTIR spectrum of EPS-1 (Fig. 4) showed a strong band at 3444.87 cm^{-1}, which was attributed to the stretching vibration of O–H in the constituent sugar residues. The band at 2927.49 cm^{-1} was associated with the stretching vibration of C–H in the sugar ring. The absorptions around 1699.00 cm^{-1} were due to the stretching vibration of C=O, and the band at 1384 cm^{-1} represented CH_2 and OH bending (Sun, Fang, Goodwin, Lawther, & Bolton, 1998). The band at 850.60 cm^{-1} was attributed to α-type glycosidic linkages, while the band at 763.97 cm^{-1} was associated with α-isomeric pyranose symmetrical stretching vibration (Cheng, Wan, Jin, Wang, & Xu, 2008). The prominent absorptions at 1144.89, 1081.47, and 1023.10 cm^{-1} also indicated the α-pyranose form of the glucosyl residue. Therefore, it can be concluded that EPS-1 belongs to the α-type heteropolysaccharide with pyran group.

3.3. Immune and antioxidant activities of EPS-1

The proliferative responses of lymphocytes to LPS and the serum TNF-α concentration in CP-treated mice reduced markedly ($p < 0.05$) compared with control animals (Table 1). Animals receiving EPS-1 along with CP showed significant recovery in splenic lymphocyte proliferation and the level of TNF-α ($p < 0.05$) compared with CP treated alone. Furthermore, treatments with EPS-1 increased the LPS-induced lymphocyte proliferation significantly ($p < 0.05$) compared with the control animals.

CP treatment caused a significant reduction in activities of antioxidant enzymes SOD and GPx in liver compared with control animals ($p < 0.05$) (Table 2). EPS-1 treatment significantly blocked the decrease in these antioxidant enzyme activities of mice treated with CP ($p < 0.05$).

Table 1
Effect of EPS-1 on immune functions.

Group	Lymphocyte proliferation ($OD_{570\,nm}$)	TNF-α (ng/mL)
Control	0.475 ± 0.020[a]	5.140 ± 0.000[a]
CP	0.269 ± 0.013[b]	2.703 ± 0.249[b]
CP + EPS-1	0.429 ± 0.004[a]	4.385 ± 0.841[a]
EPS-1	0.583 ± 0.046[c]	4.210 ± 0.156[a]

Values are expressed as means ± S.D. of six animals. Means within a column with different letters (a–c) differ significantly ($p < 0.05$). *Abbreviation*: TNF-α, tumor necrosis factor α.

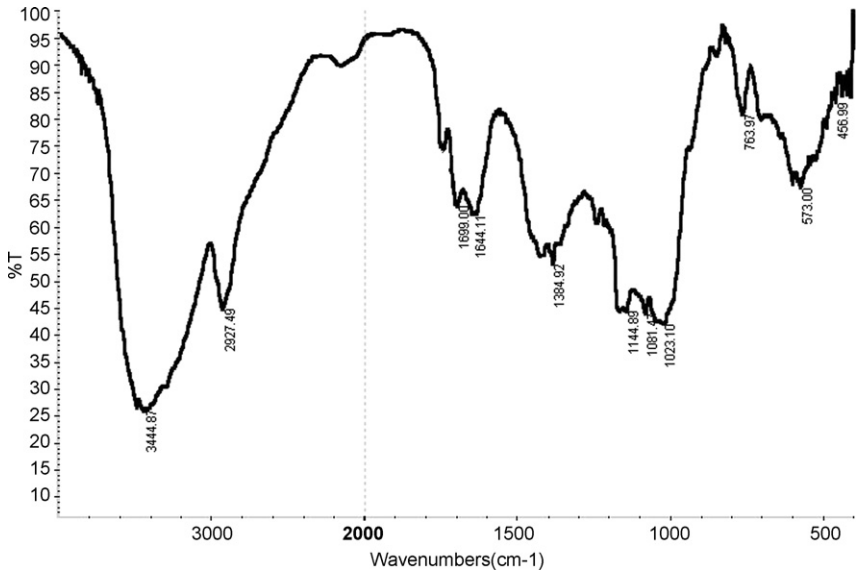

Fig. 4. FTIR spectra of the polysaccharide EPS-1.

Table 2
Effect of EPS-1 on antioxidant status in liver.

Group	SOD (U/mg prot)	GPx (U/mg prot)
Control	300.586 ± 8.021[a]	871.570 ± 35.572[a]
CP	285.186 ± 8.143[b]	709.086 ± 100.588[b]
CP + EPS-1	301.349 ± 1.887[a]	867.760 ± 10.431[a]
EPS-1	327.190 ± 6.010[c]	864.025 ± 27.672[a]

Values are expressed as means ± S.D. of six animals. Means within a column with different letters (a–c) differ significantly ($p < 0.05$). *Abbreviations*: SOD, superoxide dismutase; GPx, glutathione peroxidase.

4. Discussion

The water-soluble extracellular polysaccharide (EPS-1) was isolated from the fermentation broth of *E. cloacae* Z0206. EPS-1 was composed of glucose, mannose and galactose with a molar ratio of 6.860:1.180:0.455 and its average molecular weight was approximately 24 kDa. It was reported that the bioactive glycoprotein from *E. cloacae*, with a molecular weight of 33 kDa, was composed of xylose (Zhang et al., 2002). The partially purified polysaccharide produced by *E. cloacae* WD7 was characterized as an acidic heteropolysaccharide, composed of neutral sugars (29.4%), uronic acids (14.2%) and amino sugars (0.93%); function group analysis by FTIR spectroscopy showed the presence of hydroxyl, carboxyl, carbonyl and methoxyl groups (Prasertsan, Dermlim, Doelle, & Kennedy, 2006). These results indicate that EPS-1 produced by *E. cloacae* Z0206 has a different structure. It is noted that the characteristics of polysaccharides, i.e. sugar components, molecular weight, and the structure of the main chain and branches strongly affect the activities of polysaccharides (Jiang et al., 2008). These characteristics may endow EPS-1 with special activities.

The high molecular weight polysaccharides are often called biological response modifiers (BRM) or immunopotentiators, and it is generally accepted that they can enhance various immune responses *in vivo* and *in vitro* (Liu et al., 2007). However, immunostimulatory effects of a drug or nutritional supplement are difficult to evaluate in healthy animals (Huang, Wu, Chen, Yang, & Wang, 2007). Therefore, we made an immunosuppressive animal model to evaluate the biological activities of EPS-1.

CP is a cytotoxic alkylating agent with a high therapeutic index and broad spectrum of activities against a variety of cancers, as well as B cell malignant diseases (Sun & Peng, 2008). However, its use also introduces the possibility of immunotoxicity, teratogenicity and genotoxicity (Bin-Hafeez, Ahmad, Haque, & Raisuddin, 2001; Franke, Pra, Erdtmann, Henriques, & da Silva, 2005). CP was shown to inhibit the humoral and cellular immune responses in experimental animal models (Masnaya & Ratner, 2000; Rondinone, Giovanniello, Barrios, & Nota, 1983). In addition, studies have indicated that CP has a prooxidant character, and its administration may lead to the generation of oxidative stress in liver, lungs and serum in mice and rats with a resulting decrease in the activities of antioxidant enzymes and an increase in lipid peroxidation in these tissues (Mathew & Kuttan, 1997). Therefore, CP has been used to establish an experimental model applicable to assay the immunomodulation and antioxidant properties by antibiotics or other samples in normal or immunocompromised mice (Bujalance, Moreno, Jimenez-Valera, & Ruiz-Bravo, 2007).

Lymphocyte proliferation is a crucial event in the activation cascade of both the cellular and humoral immune responses (Xu et al., 2009). Splenic lymphocyte proliferation induced by LPS can be used to examine B lymphocyte activity (Han et al., 1998). Some cytokines such as TNF-α, interleukin-2 (IL-2) and interferon-γ (INF-γ) can induce a cell-mediated immune response and directly or indirectly regulate immune reactions (Mosmann & Coffman, 1989). TNF-α can induce the production of cytokines that could participate in the host response to infecting pathogens (Jablons et al., 1989). The present study showed that combined treatment with EPS-1 and CP significantly promoted the recovery of the lymphocyte proliferation response and the level of TNF-α in serum compared to treatment with CP alone, indicating that EPS-1 had a potent effect on the cellular immune response.

Oxidative stress is a crucial mediator of cell injury and pathology. Reactive oxygen species (ROS) can lead to the autooxidation of glycosylated protein, induction of membrane damage and oxidation of cellular lipids and proteins (Fang, Yang, & Wu, 2002). Several antioxidant enzymes, including SOD and GPx, are important buffers in the interception and degradation of superoxide anion and hydrogen peroxide. SOD is the only enzyme that disrupts superoxide radicals, and it can convert superoxide to hydrogen peroxide and maintain low superoxide concentrations (Jiang et al., 2008). GPx is an equally important antioxidant, able to react with hydrogen peroxide, thus preventing intracellular damage (Li, Zhou, & Li, 2007). According to this study, EPS-1 can promote the activities of SOD and GPx in hepatocytes, indicating it has antioxidant activity

in vivo. It can be concluded that EPS-1 treatment might ameliorate the synthesis of essential antioxidant enzymes, which have a role in preventing the pathological concentrations of oxygen radicals or improving biochemical changes induced by CP.

5. Conclusions

In summary, the present study is the first to demonstrate the isolation and biological activities of a novel exopolysaccharide, EPS-1, from the submerged culture broth of *E. cloacae* Z0206. Preliminary tests suggested that treatment with EPS-1 resulted in accelerated recovery of immuno- and antioxidant-suppression in CP-treated mice. EPS-1 significantly exhibited immunomodulatory and antioxidant activity *in vivo*. The structural and pharmacological results obtained might help increase knowledge of the relationship between the structure and biological activities of polysaccharides. However, the exact chemical structure of EPS-1 is not clear yet, and little is known regarding the essential structure responsible for its biological activity. This research will be carried out in our laboratory.

Acknowledgements

This study was supported by the National Key Technology R&D Program, China (2006BAD14B06-4) and the Science and Technology Key Program of Zhejiang Province, China (2007C12041).

References

Bin-Hafeez, B., Ahmad, I., Haque, R., & Raisuddin, S. (2001). Protective effect of *Cassia occidentalis* L. on cyclophosphamide-induced suppression of humoral immunity in mice. *Journal of Ethnopharmacology*, *75*, 13–18.

Bujalance, C., Moreno, E., Jimenez-Valera, M., & Ruiz-Bravo, A. (2007). A probiotic strain of *Lactobacillus plantarum* stimulates lymphocyte responses in immunologically intact and immunocompromised mice. *International Journal of Food Microbiology*, *113*, 28–34.

Cheng, A., Wan, F., Jin, Z., Wang, J., & Xu, X. (2008). Nitrite oxide and inducible nitric oxide synthase were regulated by polysaccharides isolated from *Glycyrrhiza uralensis* Fisch. *Journal of Ethnopharmacology*, *118*, 59–64.

Dubois, M., Gilles, K. A., Harmilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

Fang, Y. Z., Yang, S., & Wu, G. (2002). Free radicals, antioxidants, and nutrition. *Nutrition*, *18*, 872–879.

Franke, S. I., Pra, D., Erdtmann, B., Henriques, J. A., & da Silva, J. (2005). Influence of orange juice over the genotoxicity induced by alkylating agents: An *in vivo* analysis. *Mutagenesis*, *20*, 279–283.

Han, S. B., Kim, Y. H., Lee, C. W., Park, S. M., Lee, H. Y., Ahn, K. S., et al. (1998). Characteristic immunostimulation by angelan isolated from *Angelica gigas* Nakai. *Immunopharmacology*, *40*, 39–48.

Huang, G. C., Wu, L. S., Chen, L. G., Yang, L. L., & Wang, C. C. (2007). Immuno-enhancement effects of Huang Qi Liu Yi Tang in a murine model of cyclophosphamide-induced leucopenia. *Journal of Ethnopharmacology*, *109*, 229–235.

Jablons, D. M., Mule, J. J., McIntosh, J. K., Sehgal, P. B., May, L. T., Huang, C. M., et al. (1989). IL-6/IFN-β2 as a circulating hormone. Induction by cytokine administration in humans. *Journal of Immunology*, *142*, 1542–1547.

Jiang, Y. H., Jiang, X. L., Wang, P., Mou, H. J., Hu, X. K., & Liu, S. Q. (2008). The antitumor and antioxidative activities of polysaccharides isolated from *Isaria farinosa* B05. *Microbiological Research*, *163*, 424–430.

Kaspers, B., Lillehoj, H. S., Jenkins, M. C., & Pharr, G. T. (1994). Chicken interferon-mediated induction of major histocompatibility complex class II antigens on peripheral blood monocytes. *Veterinary Immunology and Immunopathology*, *44*, 71–84.

Li, X. L., Zhou, A. G., & Li, X. M. (2007). Inhibition of *Lycium barbarum* polysaccharides and *Ganoderma lucidum* polysaccharides against oxidative injury induced by γ-irradiation in rat liver mitochondria. *Carbohydrate Polymers*, *69*, 172–178.

Liu, C., Lin, Q., Gao, Y., Ye, L., Xing, Y., & Xi, T. (2007). Characterization and antitumor activity of a polysaccharide from *Strongylocentrotus nudus* eggs. *Carbohydrate Polymers*, *67*, 313–318.

Luo, D., & Fang, B. (2008). Structural identification of ginseng polysaccharides and testing of their antioxidant activities. *Carbohydrate Polymers*, *72*, 376–381.

Masnaya, N. V., & Ratner, G. M. (2000). Peculiarities of primary humoral immune response in mice with cytostatic disease. *Bulletin of Experimental Biology and Medicine*, *129*, 370–373.

Mathew, S., & Kuttan, G. (1997). Antioxidant activity of *Tinospora cordifolia* and its usefulness in the amelioration of cyclophosphamide induced toxicity. *Journal of Experimental and Clinical Cancer Research*, *16*, 407–411.

Mosmann, T. R., & Coffman, R. L. (1989). TH1 and TH2 cells: Different patterns of lymphokine secretion lead to different functional properties. *Annual Review of Immunology*, *7*, 145–173.

Prasertsan, P., Dermlim, W., Doelle, H., & Kennedy, J. F. (2006). Screening, characterization and flocculating property of carbohydrate polymer from newly isolated *Enterobacter cloacae* WD7. *Carbohydrate Polymers*, *66*, 289–297.

Rondinone, S. N., Giovanniello, O. A., Barrios, H. A., & Nota, N. R. (1983). Effect of fractional cyclophosphamide dosage on sheep red blood cell-delayed-type hypersensitivity response in mice. *Journal of Immunology*, *130*, 1600–1603.

Staub, A. M. (1965). Removal of protein—Sevag method. *Methods in Carbohydrate Chemistry*, *5*, 5–6.

Sun, R.-C., Fang, J. M., Goodwin, A., Lawther, J. M., & Bolton, A. J. (1998). Fractionation and characterization of polysaccharides from abaca fibre. *Carbohydrate Polymers*, *37*, 351–359.

Sun, H. X., & Peng, X. Y. (2008). Protective effect of triterpenoid fractions from the rhizomes of *Astilbe chinensis* on cyclophosphamide-induced toxicity in tumor-bearing mice. *Journal of Ethnopharmacology*, *119*, 312–317.

Tong, H., Xia, F., Feng, K., Sun, G., & Gao, X. (2009). Structural characterization and *in vitro* antitumor activity of a novel polysaccharide isolated from the fruiting bodies of *Pleurotus ostreatus*. *Bioresource Technology*, *100*, 1682–1686.

Wang, H., Ooi, E. V., & Ang, P. O., Jr. (2007). Antiviral polysaccharides isolated from Hong Kong brown seaweed *Hydroclathrus clathratus*. *Science in China*, *50*, 611–618.

Xu, C. L., Wang, Y. Z., Jin, M. L., & Yang, X. Q. (2009). Preparation, characterization and immunomodulatory activity of selenium-enriched exopolysaccharide produced by bacterium *Enterobacter cloacae* Z0206. *Bioresource Technology*, *100*, 2095–2097.

Zhang, M., Cui, S. W., Cheung, P. C. K., & Wang, Q. (2007). Antitumor polysaccharides from mushrooms: A review on their isolation process, structural characteristics and antitumor activity. *Trends in Food Science and Technology*, *18*, 4–19.

Zhang, M., Ren, Q., & Chen, Y. (2002). Studies on the antitumor effect of glycoproteins from *Enterobacter cloacae*. *Journal of Fudan University (Natural Science)*, *41*, 378–381, 387.