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Physicochemical properties, bioactivities and structure-activity relationships of polysaccharides from *Nostoc flagelliforme* grown under three different culture conditions

Yanru Li, Shigang Shen, Longfei Wang, Mengyuan Li, Yan Zhang, Shiru Jia, Ruiying Zhu, Peipei Han*

State Key Laboratory of Food Nutrition and Safety, Key Laboratory of Industrial Fermentation Microbiology, Ministry of Education, College of Biotechnology, Tianjin University of Science and Technology, Tianjin 300457, China

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ABSTRACT

The physicochemical properties, structural characteristics, antioxidant, radioprotective and lipid-lowering activities, as well as the underlying structure-activity relationships of polysaccharides extracted from *Nostoc flagelliforme* grown under normal (WL-EPS-1), salt stress (NaCl-EPS-1) and mixotrophic culture conditions (Glu-EPS-1) were studied. The results demonstrated that WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1 were heteropolysaccharides comprising different proportions of monosaccharides and uronic acid, with different average molecular weights of 0.99×10^3, 1.09×10^3 and 1.18×10^3 kDa, respectively. Their intrinsic viscosity were significantly different, at 24.72, 29.98, and 41.06 dL/g, respectively. The functional groups of polysaccharides were not greatly affected, but the chemical composition, triple-helix structure, chain length and surface morphology were significantly influenced by culture conditions. *In vitro* bioactivity assays showed that the antioxidant activity generally increased in the order of WL-EPS-1 < NaCl-EPS-1 < Glu-EPS-1, while NaCl-EPS-1 had the best radioprotective effect, and Glu-EPS-1 had the best lipid-lowering effect. In addition, the structure-activity relationship of the polysaccharides was analyzed by partial least squares, which revealed that the most important factors affecting the antioxidant, radioprotective and lipid-lowering activities of polysaccharides were viscosity and molecular weight. This study provides a strategy for obtaining high-bioactivity polysaccharides by appropriate regulation of culture conditions, as well as opening new directions for the molecular modification of polysaccharides.

1. Introduction

The wide array of polysaccharides used in biomedicine and the food industry exhibits antioxidant[1], anti-tumor[2], anti-coagulation[3], antiviral[4], radioprotective[5], anti-cancer[6] and immunomodulatory[7] activities. In recent years, active polysaccharides have been widely investigated by the academic community because of their good safety profile, few toxic side effects and good therapeutic effect. *Nostoc flagelliforme* is an edible cyanobacterium with excellent nutritional and herbal value, considered in traditional Chinese medicine to have the effects of clearing heat and detoxicating, coordinating intestines and stomach, as well as lowering blood pressure[8]. Polysaccharides are one of the main active components of *N. flagelliforme*, which has antioxidant, immunomodulatory, antibacterial, anti-inflammatory,

* Corresponding author at: State Key Laboratory of Food Nutrition and Safety, Key Laboratory of Industrial Fermentation Microbiology, Ministry of Education, College of Biotechnology, Tianjin University of Science and Technology, Tianjin 300457, China.
E-mail address: pphan@tust.edu.cn (P.P. Han)
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antiviral, anti-tumor and other biological effects, which gives it good development and application prospect[9-10].

The bioactivities of polysaccharides are determined by their physicochemical properties, including molecular weight, monosaccharide composition, glycosidic bonds and their configuration, as well as the main-and side-chain conformations[11]. In some instances, the physicochemical properties of polysaccharides were found to depend on the culture conditions of the producing strain. Lu et al.[12] found that salt stress affected the ratio of constituent monosaccharides in *N. flagelliforme* polysaccharides. When *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772 was cultured in media with different carbon sources, the proportions of constituent monosaccharides of its exopolysaccharide (EPS) was also different[13]. Similarly, the polysaccharides synthesized by *Lactobacillus rhamnosus* E/N cultured with five carbon sources had different molecular mass distributions, chain length, branching, and thickness. Furthermore, a correlation was observed of the viscosity of EPS solutions with their molecular mass ratio and polymer chain thickness[14]. Shen et al.[15] reported that the antioxidant activity, proportion of constituent monosaccharides, average molecular weight and microstructure of polysaccharides synthesized under different culture conditions were different. Therefore, it is possible to produce tailored polysaccharides with high bioactivity by regulating the culture conditions.

Enhancing the bioactivity of polysaccharides from microalgae can expand their potential applications in the fields of pharmaceuticals or nutrition. Therefore, the strategy to improve the bioactivity of polysaccharides by adjusting the culture conditions was proposed. In our previous work, it was found that different culture conditions significantly affected the antioxidant activity of *N. flagelliforme* capsular polysaccharides (CPS)[15]. However, EPS fractions and other bioactivities have not been studied in depth. In addition, there is still a paucity of research on the relationships between the bioactivity, structure, and physiochemical properties of polysaccharides as well as the factors that regulate *N. flagelliforme* culture. As the study of the structure-activity relationship of polysaccharides is the basis of their application in the food or pharmaceutical industry, it is of great significance to improve the bioactivity of known polysaccharides by molecular modification. This study aimed to improve the bioactivity of polysaccharides by regulating the culture conditions, as well as to identify the key factors that may affect their bioactivity by partial least squares (PLS) analysis. The results showed that the polysaccharides extracted from *N. flagelliforme* under salt stress and mixotrophic culture conditions had better antioxidant, radioprotective and lipid-lowering activities, whereby the viscosity and molecular weight were the key influencing factors. These results indicated that the change of fermentation conditions greatly affects the bioactivity of polysaccharides. Therefore, the fermentation conditions could be properly regulated to obtain the required high-bioactivity polysaccharides. This work provides useful information for further development and application of cyanobacterial polysaccharides in the food and pharmaceutical industries.

2. Materials and methods

2.1 Strains, cell lines, media and culture conditions

N. flagelliforme (TCCC11757) was provided by the Tianjin Key Laboratory of Industrial Microbiology (Tianjin, China). The cells were pre-cultured in 500 mL Erlenmeyer flasks containing 200 mL BG-11 medium under continuous illumination of 60 μmol/(m^2·s) at 25 ℃ for 10 days, after which they were used to inoculate (10%, *V*/*V*) a 80 L airlift photo-bioreactor with 60 L of BG-11 medium, incubated at 25 ℃ and pH 8.0 for 24 days, which was set as the control group. The other two groups of cells were first cultured in BG-11 medium for 15 days, after which NaCl (salt stress group, 0.5 mol/L) or glucose (Glc; mixotrophic culture group, 4 g/L) was added, followed by continued culture for 9 days. The aeration rate was set to 48 L/min and the light intensity was 60 μmol/(m^2·s), which was provided by 8 fluorescent lamps set into the bioreactor.

RAW246.7, AML-12 and HepG2 cells from the American Type Culture Collection (ATCC, Manassas, VA) were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, USA) containing 10% fetal bovine serum (FBS; AusGeneX, Brisbane, Australia), streptomycin and penicillin (100 U/mL, Solarbio, Beijing, China) at 37 ℃ in an incubator with 5% CO_2.

2.2 Characterization of polysaccharides

2.2.1 Preparation and purification of polysaccharides

The *N. flagelliforme* culture was centrifuged at 10 000 × *g* for 10 min at 4 ℃ to harvest the culture supernatant, which was directly concentrated by ultrafiltration to 10% of the original volume. The concentrate was combined with 4 volumes of ethanol and kept overnight at 4 ℃. Crude EPS was collected by centrifugation and lyophilization, and the polysaccharide yield per unit volume (mg/L) was calculated after weighing. Then, the dried EPS was redissolved in deionized water and further purified according to a previously described method[16]. Briefly, the solution was loaded onto a DEAE-650M cellulose anion exchange column (2.6 cm × 60 cm; TOSOH Corp., Japan). The column was eluted with deionized water first, and then with a linear gradient of 0–1.0 mol/L NaCl solution. The main fractions were collected and loaded onto a Sephadex G100 gel chromatography column (1.6 cm × 80 cm; Pharmacia Corporation, Sweden) and eluted with deionized water. Finally, three polysaccharide fractions named WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1 were obtained.

2.2.2 Chemical analysis

The protein contents was measured using a BCA protein quantitative reagent kit (Solarbio, Beijing, China). The Folin-Ciocalteu[17] and sulfuric acid carbazole colorimetric methods[18] were used to determine the content of total phenols and uronic acids, respectively.

The monosaccharide compositions of polysaccharides was analyzed according to a previously reported method[19].

The homogeneity and molecular weight of polysaccharides was determined by high performance size exclusion chromatography (HPSEC) using a TSK-Gel G4000 PWXL column (30 cm × 7.5 mm) kept at 35 ℃, on a system equipped with a refractive index detector. The samples comprising 1 mg/mL polysaccharides were filtered through a 0.22 μm pore-size membrane. Then, 20 μL of each polysaccharide solution and the dextran standards (1 mg/mL) were injected and separated using ultrapure water (Milli-Q) as the mobile phase at a flow rate of 0.5 mL/min at 35 ℃. The molecular weight of the polysaccharide samples was calculated based on the retention times compared to those of the dextran standards.

2.2.3 Analysis of intrinsic viscosity

The intrinsic viscosity of the polysaccharide samples was measured according to a previously reported method[20]. Briefly, 15 mL of the polysaccharide solution (0.1 g/L) was added to the Ubbelohde capillary tube (Φ = 0.5 mm, Equity Instrument Factory, Shanghai, China) in a 25 °C thermostat-controlled water bath cauldron, and the descent time (t) of polysaccharide solution flowing through the capillary tube was recorded with a stopwatch. Distilled water was used to determine t_0. The relative viscosity (η_r) was calculated as $\eta_r = t/t_0$ and the specific viscosity (η_{sp}) was calculated as $\eta_{sp} = (t - t_0)/t_0$. The intrinsic viscosity (η) was determined using the following equation:

$$\eta = \frac{\eta_{sp} + 5\ln\eta_r}{6c}$$

Where c is the concentration of the polysaccharide solution (g/L).

2.2.4 Ultraviolet (UV) spectra

According to our previous report[21], each polysaccharide sample solution (1 mg/mL) was analyzed by UV spectroscopy on a UV-2401 ultraviolet spectrophotometer (Shimadzu Corp., Japan) in the wavelength range of 200–400 nm.

2.2.5 Fourier transform infrared (FT-IR) spectra

According to our previous report[21], the sample (1 mg) was ground with dried KBr (150 mg) and pressed into pellets for FT-IR measurement (Bruker Corporation, Germany) in the wavenumber range of 400–4 000 cm^{-1}.

2.2.6 Congo red experiment

The triple-helix structure of polysaccharides was analyzed based on their interaction with Congo red. Briefly, 1 mL of polysaccharide solution (1 mg/mL) was mixed with 1.0 mL Congo red (80 μmol/L), followed by the addition of NaOH into the solution to a final concentration of 0, 0.1, 0.2, 0.3, 0.4, or 0.5 mol/L. The mixture was left to stand for 10 min in the dark, followed by spectroscopic scanning to record the maximum absorption wavelength (λ_{max}).

2.2.7 Analysis of molecular surface morphology

The sample coated with a thin gold layer was placed on the substrate. The morphology of polysaccharide samples was observed using a scanning electron microscope (SEM; FEI Company, the Netherland).

The polysaccharide sample solution (5 μL, 10 μg/mL) was pipetted onto a freshly cleaved mica substrate and dried at room temperature. The surface morphology was scanned via atomic force microscopy (AFM) in tapping mode (Bruker Corporation, Germany).

2.3 Antioxidant activity assays

2.3.1 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS) cation radical scavenging activity

ABTS cation radical scavenging activity was determined according to a previously reported method[22]. Briefly, ABTS cation radical solution was produced by mixing ABTS aqueous solution (final concentration 7.4 mmol/L) (Solarbio, Beijing, China) with $K_2S_2O_8$ solution (final concentration 2.6 mmol/L), followed by incubation in the dark at room temperature for 16 h. Immediately prior to use, the ABTS cation radical solution was diluted with deionized water to an absorbance at 734 nm of 0.70 ± 0.02. Then, 100 μL of different concentrations (0.5–2.5 mg/mL) of the polysaccharide solution or ascorbic acid (VC) (positive control) (Tianjin Taixin Technology Co., Ltd., Tianjin, China) were mixed with 600 μL of the ABTS cation radical solution and placed in the dark for 6 min prior to measuring the absorptance at 734 nm.

2.3.2 Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was dtermeined according to a previously reported method[15]. Briefly, 1 mL of different concentrations (0.5–2.5 mg/mL) of polysaccharide sample or VC (positive control) were mixed with 1 mL (5 mmol/L) salicylic acid-ethanol, 1 mL $FeSO_4$ (5 mmol/L), and 1 mL H_2O_2 (5 mmol/L), followed by incubation at 37 °C for 30 min, after which the absorbance at 510 nm was measured.

2.3.3 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity was determined according to a previously reported method[15]. Briefly, 3 mL samples comprising polysaccharides or VC (positive control) of different concentrations (0.5–2.5 mg/mL) and 1 mL DPPH-ethanol solution (0.1 mmol/L DPPH in 95% ethanol) were incubated in the dark at 37 °C for 30 min, after which the absorbance at 517 nm was measured.

2.4 Cell viability assay

Cell viability was examined using the MTT assay (Solarbio, Beijing, China). The cells were seeded into 96-well plates and grown to 70% confluence. After treatment with polysaccharides (0, 25, 50, 100, or 200 μg/mL final concentration) for 24 h, the cells were incubated with 5 mg/mL MTT solution (20 μL per well) for another 4 h. The medium was then removed and 100 μL of dimethyl sulfoxide (DMSO) was added to each well to solubilize the formazan crystals, after which the absorbance at 490 nm was measured using a microplate reader.

2.5 Radioprotective ability assay

AML-12 cells (0.5×10^5 cells/mL in 96-well plates) were cultured overnight, first pretreated with polysaccharides (0, 25, 50, 100, or 200 μg/mL final concentration) for 12 h and then irradiated with a dose of 4.0 Gy radiation at 2.0 Gy/min using a ^{60}Co γ irradiator at the Institute of Isotope Research, Henan Academy of Sciences (Zhengzhou, China)[23], after which culture was continued for 24 h. Finally, the MTT assay described above was used to determine cell viability.

2.6 Lipid-lowering activity assay

HepG2 cells were cultured in 6-well plates for 24 h, and then treated with polysaccharide solution or atorvastatin calcium tablets

(PC group) (Pfizer Pharmaceuticals, Ltd., USA) at different concentrations (50, 100, 200 μg/mL), followed by incubation with 4 μg/mL oleic acid (Sigma, St. Louis, USA) for 48 h. The control group (CON) consisted of HepG2 cells without oleic acid supplementation. The model group (MC) comprised HepG2 cells treated solely with oleic acid. Finally, the cells were collected and lysed on ice, after which the total cholesterol (TC) and triglyceride (TG) levels were measured according to the instructions of the respective kits (Nanjing Jiancheng Biotechnology Co., Ltd., Nanjing, China).

2.7 Statistical analyses

The data were analyzed using GraphPad Prism 5.0 (Graph Pad Software Inc., San Diego, CA, USA). Data are presented as means ± standard deviation (SD) and comparisons between groups were performed using Student's *t*-test.

3. Results and discussion

3.1 Extraction and purification

To compare the polysaccharide-producing capacities of *N. flagelliforme* grown under three different culture conditions, the EPS production was calculated as presented in Fig. S1. After 24 days of culture, the EPS productions of the salt stress group and mixotrophic culture group were increased by 101.5% and 300.6% compared with the control group ($P < 0.01$ and $P < 0.001$, respectively), indicating that both salt stress and mixotrophic culture conditions could significantly increase the EPS yield. The crude EPS was collected by lyophilization and then redissolved in deionized water for further purification. The elution curve of crude EPS on cellulose DEAE-650M is illustrated in Fig. 1A. No polysaccharide signal was detected when eluted with deionized water, while a single fraction (control and NaCl stress groups) or two fractions (mixotrophic culture group) were obtained in an elution with an NaCl gradient of 0.1–1.0 mol/L. The main fractions named WE-1, NE-1 and GE-1 were collected (Fig. 1A). The three fractions WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1 were further purified by HPSEC on a Sephadex G-100 column (Fig. 1B). The purified fraction was collected, concentrated, dialyzed, and freeze-dried for subsequent analysis.

3.2 Characteristics of polysaccharides

3.2.1 Chemical properties, UV absorption spectra, molecular weight, intrinsic viscosity and monosaccharide composition

According to the analysis, Glu-EPS-1 had the highest content of total sugar (77.17%) and uronic acids (13.83%). The contents of total sugar (70.31%) and uronic acids (8.98%) were the lowest in WL-EPS-1. No phenol was detected in any of the polysaccharide samples (Table 1). Furthermore, the results of full UV wavelength scan showed no pronounced absorbance peaks at 260 and 280 nm (Fig. 2A), confirming that the polysaccharide samples contained no significant protein or nucleic acid impurities.

Table 1
Chemical and monosaccharide composition of WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1.

Basic physicochemical properties	WL-EPS-1	NaCl-EPS-1	Glu-EPS-1
Chemical composition (%, *m*/*m*)			
Total sugar	70.31 ± 3.92	73.17 ± 2.85	77.17 ± 4.28
Protein	0.43 ± 0.03	0.49 ± 0.02	0.50 ± 0.02
Uronic acids	8.98 ± 0.72	11.26 ± 1.04	13.83 ± 1.29
Total phenols	ND	ND	ND
Monosaccharide composition (%, *n*/*n*)			
Xylose (Xyl)	0.68 ± 0.02	1.26 ± 0.05	0.88 ± 0.03
Arabinose (Ara)	1.41 ± 0.01	1.43 ± 0.03	1.12 ± 0.07
Ribose (Rib)	2.71 ± 0.11	2.12 ± 0.06	2.80 ± 0.10
Rhamnose (Rha)	3.25 ± 0.10	5.80 ± 0.07	3.69 ± 0.28
Fucose (Fuc)	0.97 ± 0.04	3.33 ± 0.26	1.91 ± 0.10
Mannose (Man)	22.46 ± 0.23	24.50 ± 0.44	22.48 ± 0.43
Galactose (Gal)	25.05 ± 0.10	23.88 ± 0.37	22.25 ± 0.51
Glc	38.75 ± 0.23	31.77 ± 0.22	40.27 ± 0.31
Glucuronic acid (GlcA)	3.72 ± 0.03	5.91 ± 0.18	4.78 ± 0.19

Note: ND, not detected.

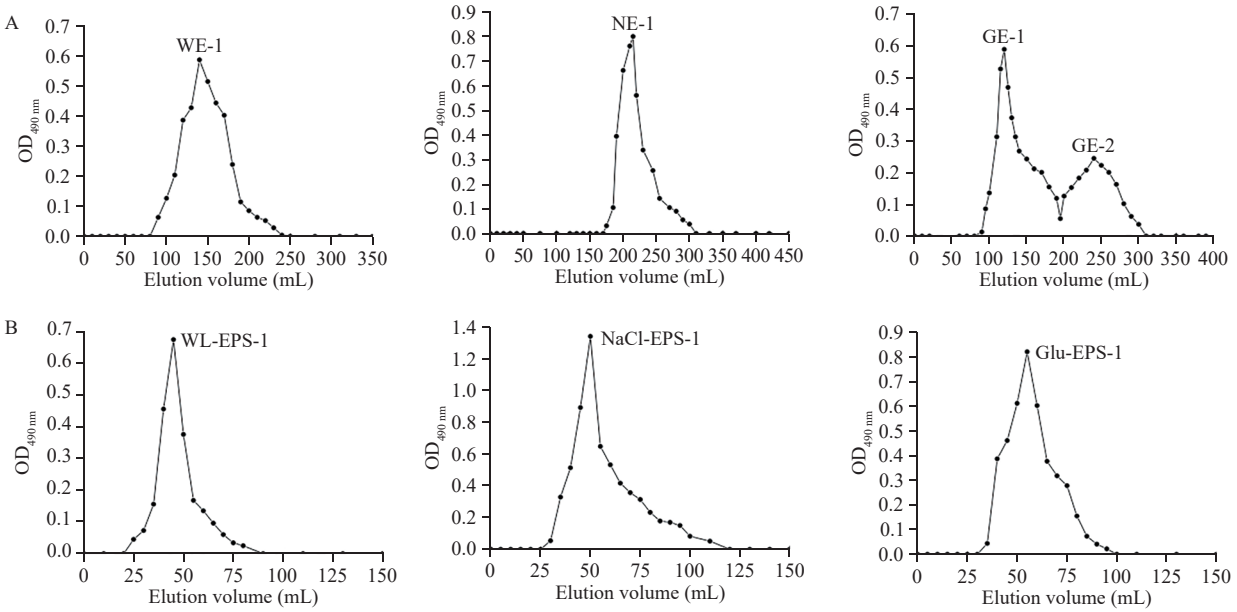

Fig. 1 Elution curves of *N. flagelliforme* polysaccharides on (A) DEAE-650M and (B) Sephadex G-100 columns.

Moreover, the HPSEC profile showed a single and symmetrical peak, indicating that each kind of polysaccharide was homogeneous (Fig. S2). The average molecular weights of WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1 were estimated to be 0.99×10^3, 1.09×10^3 and 1.18×10^3 kDa, respectively.

The intrinsic viscosity is the specific viscosity of a polymer solution with a concentration approaching zero. Notably, its value does not change with the concentration of the solution, as it is mainly depending on the relative molecular weight of the polymer, chain rigidity and solvent type. As shown in Fig. 2B, the intrinsic viscosity of WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1 in deionized water were 24.72, 29.98, and 41.06 dL/g, respectively. These results indicate that the altered fermentation conditions affected the intrinsic viscosity of polysaccharides, which may be due to the extension of polysaccharide structure under different fermentation conditions, leading to an increase of viscosity in aqueous solution.

The monosaccharide compositions of the polysaccharide samples were shown in Fig. S3 and Table 1, WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1 were heteropolysaccharides mainly composed of Glc, Man, Gal, Rha and GlcA, with molar ratios of 38.75:22.46:25.05:3.25:3.72, 31.77:24.50:23.88:5.80:5.91 and 40.27:22.48:22.25:3.69:4.78, respectively. In the monosaccharide composition of WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1, the contents of Glc, Man and Gal were similar, while those of Fuc and GlcA varied considerably.

3.2.2 FT-IR spectra

The main functional groups and chemical bonds of polysaccharide samples can be analyzed using FT-IR spectroscopy. The FT-IR spectra of WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1 are displayed in Fig. 2C. All polysaccharide samples had a strong characteristic absorption peak near 3 421 cm^{-1} and a weak peak around 2 924 cm^{-1}, which were attributed to the stretching vibrations of –OH and C–H bonds, respectively[21]. Furthermore, the two peaks at 1 640 and 1 413 cm^{-1}, characteristic of carboxylic groups suggested the presence of uronic acids in the polysaccharides[24]. The absorption peaks at 1 035 and 1 140 cm^{-1}, which spanned from 1 200 to 1 000 cm^{-1}, were attributed to the bending vibrations of C–O–C and C–O–H groups, indicating the presence of pyranose sugars[25-26]. In addition, the signals at 899 cm^{-1} found in WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1 was ascribed to a β-type glycosidic linkage[27], while the peak near 786 cm^{-1} is likely due to the presence of *D*-glucopyranose rings[28]. Another obvious absorption peak at 612–631 cm^{-1}, indicative of C–H out-of-plane flexural vibrations[29], was also found in all samples.

3.2.3 Triple-helix structure of the polysaccharides

Polysaccharides with a triple-helix conformation can form a complex with Congo red. It had been reported that the λ_{max} of the complex of polysaccharides with this conformation shifts to a longer wavelength at low concentrations and is then gradually reduced to reach a constant value at increasing NaOH concentrations[30]. Fig. 2D shows the maximum absorption wavelengths of WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1 Congo red complexes at various NaOH concentrations. The λ_{max} of WL-EPS-1 and Glu-EPS-1 increased significantly when the concentration of NaOH was increased from 0 to 0.2 mol/L, indicating that WL-EPS-1 and Glu-EPS-1 have a triple-helix structure under weakly alkaline conditions. With the increase of the NaOH concentration, the λ_{max} decreased sharply, indicating that the intramolecular hydrogen bonds were destroyed, and the triple helix structure disintegrated into single strands, which could no longer form a complex with Congo red. However, the specific trends with no red-shift and no significant decrease at NaOH concentrations in the range 0–0.5 mol/L suggested that NaCl-EPS-1 had no triple-helix structure.

3.2.4 Morphology of the polysaccharides

The morphology of WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1 was characterized by SEM (Fig. 2E). WL-EPS-1 mainly exhibited loose fragmentary aggregation and NaCl-EPS-1 showed lamellar accumulation with a relatively smooth surface, which intuitively illustrates the amorphous structure of the polysaccharide. For Glu-EPS-1, it was rod-shaped and had many small particles on its surface, which may be related to specific changes of physicochemical properties, molecular weight and structure under different fermentation conditions.

AFM is a powerful technique for directly observing the conformation of individual macromolecules. As shown in Fig. 2E, the 2D and 3D images of polysaccharide samples displayed irregular globular or linear structures with different sizes. According to the analysis, the height of WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1 about 1.3–3.5,

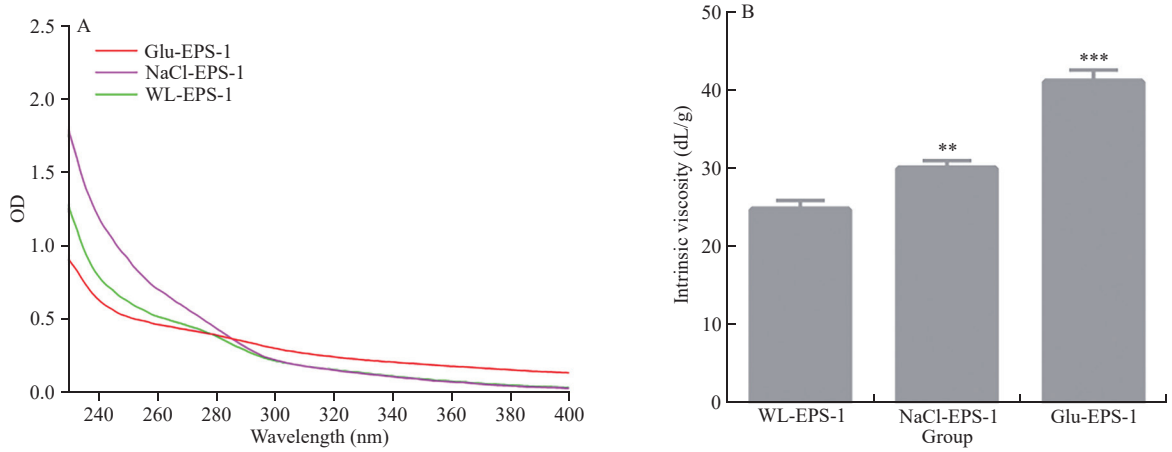

Fig. 2 (A) UV spectra, (B) intrinsic viscosity, (C) FT-IR spectra, (D) triple-helix structure, and (E) morphology of WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1. $^{**}P < 0.01$, $^{***}P < 0.001$ *vs*. WL-EPS-1. $n = 3$.

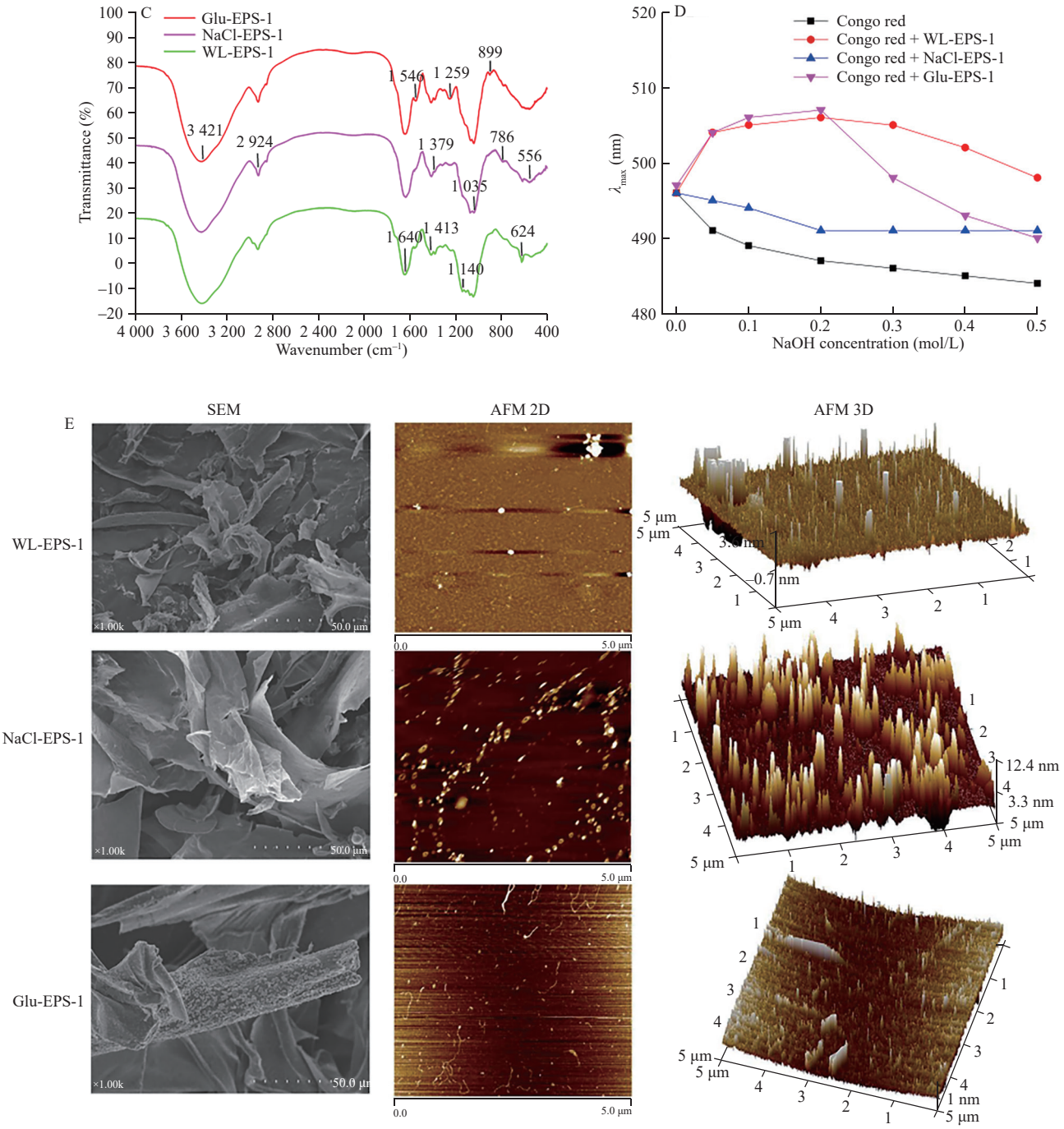

Fig. 2 (*Continued*)

3.3–12.4, and 0.6–1.3 nm respectively, which is larger than that of a single polysaccharide chain of 0.1–1.0 nm. This suggests that the observed polysaccharide samples are not a single chain, but aggregates formed by many branches of the polysaccharide intertwined with each other.

In conclusion, the culture conditions significantly affected both the basic physicochemical properties and morphological characteristics of the polysaccharides, but did not significantly affect their functional groups and conformation. It was reported that different carbon sources had no significant effect on the monosaccharide composition of EPS synthesized by *Pleurotus pulmonarius*, but had a certain effect on EPS yield and molar ratio of monosaccharides[31]. Zhao et al.[32] found that the molar ratio of monosaccharides and surface morphology of EPS obtained via fermentation from different carbon sources (Glc or sucrose) are different, but their molecular weight, functional groups and chemical bonds are not significantly different. Salt stress can increase the yield of EPS, change the ratios of constituent monosaccharides and improve its antioxidant activity[33]. Thus, our results were in good agreement with previous reports. However, the factors that regulate the composition of polysaccharides and control

their length are still an open question. One explanation could be the complex pathways involved in sugar metabolism. The synthesis of heteropolysaccharides can be divided into three steps: assimilation of simple sugars and their conversion into nucleotide derivatives; assembly of polysaccharide subunits attached to a lipid transporter, probably undecaprenyl phosphate or isoprenoid phosphate; and the elongation, translocation and polymerization of the repeating units of the polysaccharide; and finally, the secretion of the polymer into the extracellular environment[14,34]. Liu[35] found that the molecular weight, homogeneity and monosaccharide composition of EPSs obtained using different carbon source, nitrogen source, culture temperature or accelerant conditions were significantly different. The author speculated that these culture factors directly or indirectly affected the polysaccharide synthesis process, and regulated the pathway of polysaccharide anabolism through the influence of enzymes or regulatory factors related to polysaccharide synthesis in the cell, thus changing the type, composition and properties of EPSs. And in our previous work, it was found that different culture conditions significantly affected the key enzyme controlling polysaccharide synthesis, resulting in different composition of monosaccharides[19]. Therefore, it might be hypothesized that culture conditions may control the composition and chain length of polysaccharides by affecting key enzymes or regulatory factors related to polysaccharide synthesis, thus further affecting the physicochemical properties of polysaccharides[15]. However, the elucidation of the specific regulatory mechanisms still needs to be further explored based on the polysaccharide synthesis pathway.

3.3 *In vitro bioactivity of polysaccharides*

3.3.1 *In vitro antioxidant activity*

In this study, the antioxidant activity of polysaccharide samples obtained under different culture conditions was evaluated using ABTS cation, DPPH and hydroxyl radical scavenging assays, as the radical scavenging ability of the samples is closely related to their antioxidant capacity.

As shown in Fig. 3, the scavenging efficiency of WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1 exhibited a dose-effect relationship within a certain concentration range. With the increase of the concentration of polysaccharides, the scavenging efficiency for all three radicals gradually increased, but it was lower than that of VC. When the concentration of polysaccharides reached 2.5 mg/mL, the scavenging abilities of WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1 for ABTS cation, DPPH and hydroxyl radicals were 88.87%, 42.27% and 57.65%; 90.54%, 50.85% and 63.33%; and 95.23%, 52.33% and 88.43%, respectively. The results showed that the polysaccharide samples exhibited strong scavenging activity on ABTS cation and hydroxyl radicals and moderate scavenging activity on DPPH radicals. Overall, the radical scavenging ability was in the order of WL-EPS-1 < NaCl-EPS-1 < Glu-EPS-1.

Studies have shown that the antioxidant activity of polysaccharides is related to many factors, including monosaccharide composition, uronic acid content, molecular weight, and protein content[36-38]. Multiple linear regression (MLR) and artificial neural network (ANN) models showed that the contents of Ara, galacturonic acid (GalA), Fuc, Rha, uronic acid and proteins had a great effect on the DPPH and hydroxyl radical scavenging activitives of polysaccharides[39]. In this study, the content of Ara and Rha in each polysaccharide sample was roughly the same, but the content of Fuc in Glu-EPS-1 and NaCl-EPS-1 was much higher than in WL-EPS-1. In addition, Glu-EPS-1 had the highest uronic acid content among the three polysaccharides (Table 1). It has been reported that polysaccharides with relatively high molecular weight exhibit stronger biological activity[30]. The molecular weight of Glu-EPS-1 was the largest among the three polysaccharides. In general, our results were basically consistent with the above reports, and it can be inferred from this study that the differences in the antioxidant activity of polysaccharides might be attributed to the differences in the Fuc and uronic acid content, as well as the molecular weight.

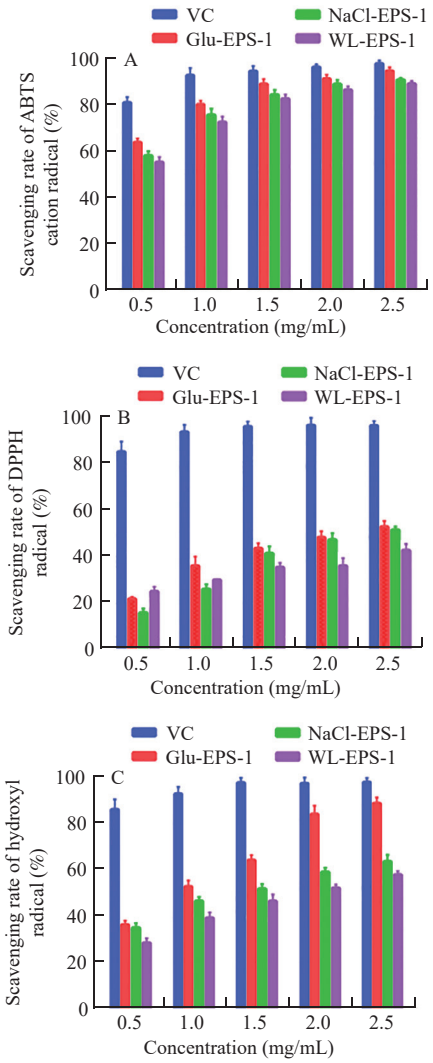

Fig. 3 Antioxidant activities of WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1. (A) ABTS cation radical scavenging activity. (B) DPPH radical scavenging activity. (C) Hydroxyl radical scavenging activity.

3.3.2 *In vitro radioprotective activity*

Ionizing radiation can induce the generation of large amounts of reactive oxygen species (ROS), while our experiments have shown that WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1 had significant antioxidant

activities, so we speculated that they also may have a certain radioprotective effect.

To detect whether WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1 affected cell survival and growth, AML-12 cells were treated with the polysaccharide preparations at different concentrations (0–200 μg/mL) for 24 h, followed by relative viability using the MTT assay. As shown in Fig. 4A, the relative viability of AML-12 cells increased with the concentration of polysaccharides, but tended to decrease when the concentration reached a certain level. Among them, the relative viability of cells treated with NaCl-EPS-1 and Glu-EPS-1 increased in the concentration range of 0–50 μg/mL, reaching 1.17 and 1.11, respectively. When the concentration was increased to 50–200 μg/mL, the relative viability of the cells decreased to 1.12 and 1.03, respectively. Similarly, the relative viability of cells treated with WL-EPS-1 increased to 1.06 in the concentration range of 0–100 μg/mL. In conclusion, the polysaccharide samples were not only non-toxic to AML-12 cells, but also promoted cell growth. Based on these results, the *in vitro* radioprotective activity of WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1 to AML-12 cells was then determined.

As shown in Fig. 4B, with increasing concentrations of WL-EPS-1 and NaCl-EPS-1, the radioprotective activity also increased. At the concentration of 200 μg/mL, the relative cell viability reached 1.08 and 1.13, respectively. When the concentration of Glu-EPS-1 was 25 μg/mL, the radioprotective ability was the highest, reached 1.09. As the polysaccharide concentration continued to increase, the relative cell viability first rapidly decreased and then slowly increased. Based these results, it was found that the polysaccharide extract obtained from *N. flagelliforme* grown under salt stress (NaCl-EPS-1) had the best radioprotective effect.

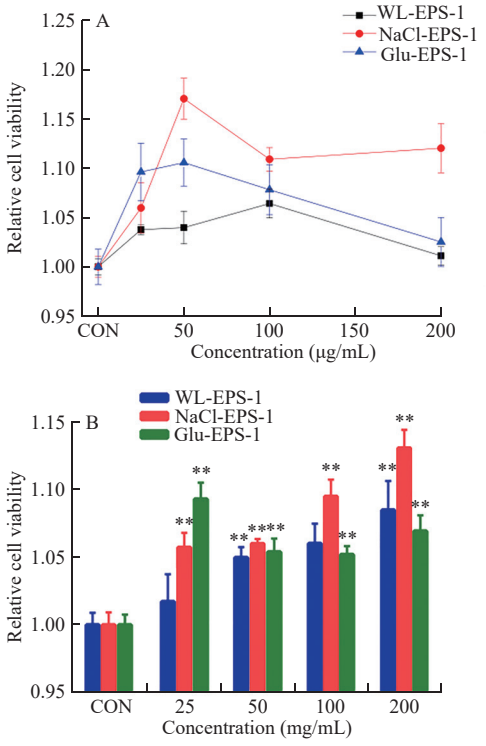

Fig. 4 Radioprotective activities of WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1. (A) Effect of WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1 on the viability of AML-12 cells. (B) Radioprotective ability of WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1. $^{**}P < 0.01$ *vs.* control. $n = 3$.

At present, the research on the mechanism of radioprotective substances mainly focuses on three aspects, whereby some radioprotective substances act as absorbers of rays[40], some act as signal molecules that can induce the self-repair function of cells by regulating the activity of related enzymes[41], while many radioprotective substances can directly remove the large number of free radicals produced by irradiation[42]. According to the *in vitro* results discussed above, Glu-EPS-1 had the highest antioxidant activity, but its radioprotective effect was weaker than that of NaCl-EPS-1. A previous study showed that VC had higher antioxidant activity *in vitro* than EPS from *Pantoea agglomerans* KFS, but its radioprotective activity was weaker than that of EPS. The author speculated that EPS can not only remove free radicals generated by UV irradiation, but also regulate the activity of related enzymes, so as to achieve better UV protection[43]. Therefore, it is speculated that polysaccharides not only protect cells by removing a large number of ROS produced by irradiation, but also may contribute to the two other radioprotective mechanisms mentioned above, resulting in different effects due to differences in polysaccharide structure, which needs to be studied further in the future.

3.3.3 *In vitro lipid-lowering activity*

Antioxidants have been shown to be an effective means of preventing and treating non-alcoholic fatty liver disease by enhancing the organism's antioxidant defense system, thereby reducing liver function damage related to lipid peroxidation. Here, we used oleic acid-induced HepG2 cells to establish a hepatic steatosis cell model *in vitro*, which was used to assess the potential lipid-lowering activities of the polysaccharide preparations.

HepG2 cells were treated with the polysaccharides at different concentrations for 24 h, and their effects on cell viability were determined using the MTT assay. As shown in Fig. 5A, the relative viability of cells treated with each polysaccharide remained above 90% at lower concentrations (25 μg/mL). When the concentration of polysaccharides increased from 25 to 200 μg/mL, the relative viability of cells treated with WL-EPS-1 and NaCl-EPS-1 remained basically unchanged, while that of Glu-EPS-1 remained above 80%. Thus, Glu-EPS-1 had a greater effect on cell viability, but the difference was considered acceptably small. Therefore, polysaccharide concentrations of 50, 100 and 200 μg/mL were selected for subsequent experiments.

The lipid lowering ability of polysaccharides was evaluated by detecting the changes of TC and TG contents in HepG2 cells after treatment. As shown in Figs. 5B and C, compared with the control group, the intracellular TC and TG contents in the MC group were increased by 6.03- and 6.12-folds, respectively, indicating that the model was successfully induced by treating HepG2 cells with OA. Compared with the MC group, the intracellular TC and TG contents in each polysaccharide treatment group significantly decreased ($P < 0.05$), and the reduction was dose-dependent. Among them, the intracellular TC and TG content of the Glu-EPS-1 group was close to that of the control group, and the lipid lowering effect was most significant.

Many studies have shown that the blood lipid-lowering effect of polysaccharides is closely related to their antioxidant capacity[44-48]. Other studies have reported that that higher molecular weight results in higher viscosity of polysaccharides, which also have a better effect on reducing blood lipids[49-50]. The results showed that

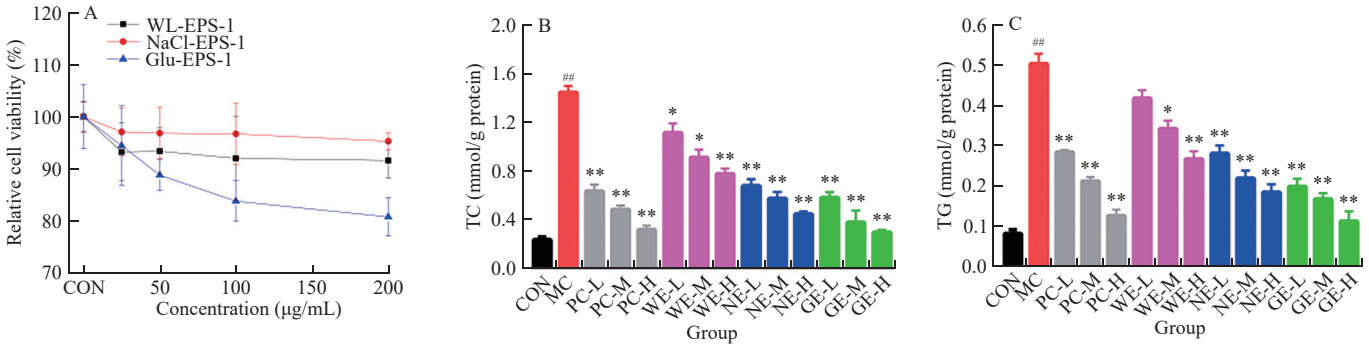

Fig. 5 Lipid-lowering activities of WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1. Effect of WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1 on the viability (A), TC contents (B) and TG contents (C) of HepG2 cells. $^{\#\#}P < 0.01$ *vs*. control; $^{*}P < 0.05$, $^{**}P < 0.01$ *vs*. the MC group. $n = 3$.

Glu-EPS-1 with the highest antioxidant activity also had the highest lipid-lowering activity. In addition, Glu-EPS-1 also had the highest molecular weight among the three, which is in good agreement with the above reports.

3.4 Structure-activity relationship of polysaccharides

PLS analysis is a regression modeling method that studies multi-dependent versus independent variables. In this study, a PLS model was used to statistically analyze the structure-activity relationship between the properties, structural characteristics (molecular weight and viscosity, total sugar, uronic acid, Glc, Ara, Rib, Rha, Gal, Fuc, Man, GlcA and Xyl content) and bioactivity (antioxidant, radioprotective and lipid-lowering activitives) of the three polysaccharide preparations obtained under different fermentation conditions.

3.4.1 Structure-antioxidant activity relationship

The PLS score plots of WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1 are shown in Fig. 6A. The sample points of the 3 polysaccharides were far away from each other, indicating that there were obvious differences in antioxidant activities among them.

The blue dots in the loading plots represent the antioxidant activity of the polysaccharide samples (DPPH and hydroxyl radical scavenging activity), and the green dots represent their properties or structural characteristics. The farther the point from the coordinate origin, the greater its contribution to the antioxidant activity of the polysaccharide samples. As shown in Fig. 6B, the viscosity of polysaccharide samples is the point farthest from the origin, while the points represented by monosaccharides with relatively small percentages among all monosaccharides mostly clustered together and were particularly close to the origin. These results indicated that the viscosity contributed the most to the antioxidant activity of polysaccharides.

Through the variable importance in projection (VIP) analysis under the PLS model, the properties or structural characteristics of *N. flagelliforme* polysaccharides could be ranked according to their contributions to the antioxidant activity, and variables with VIP values greater than 1 or 0.5 were generally selected. As shown in Fig. 6C, the variables with VIP > 1 were viscosity, molecular weight, uronic acid and total sugar, in descending order.

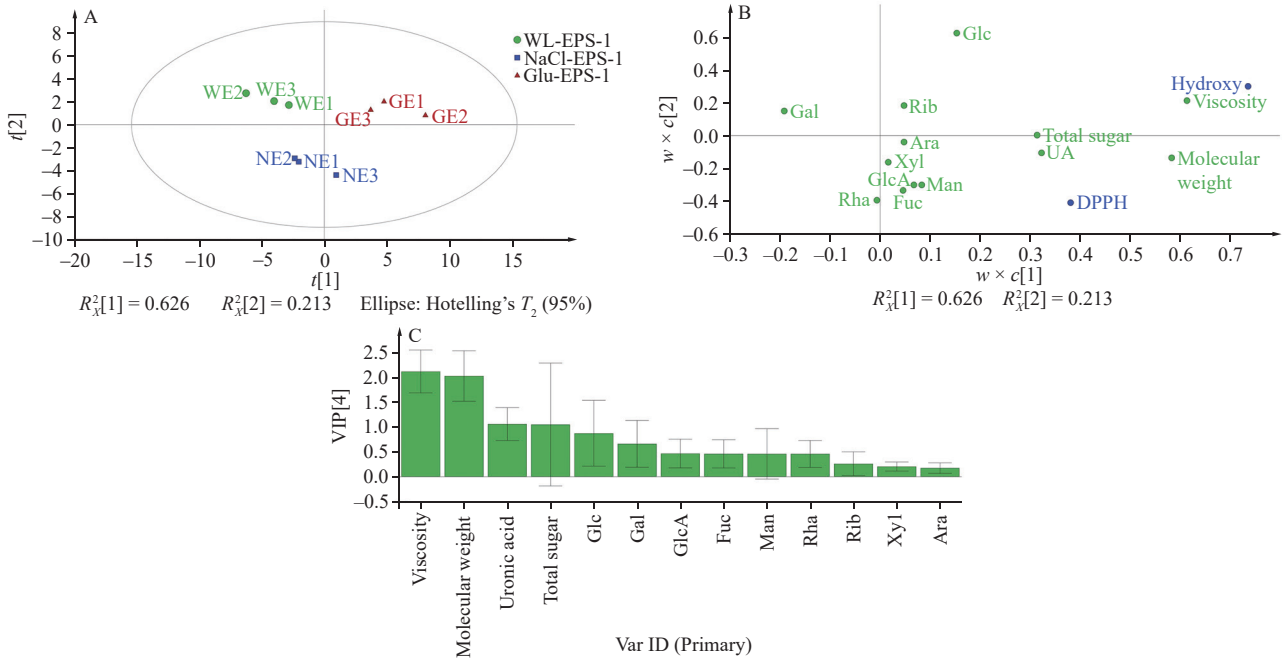

Fig. 6 (A) PLS score plots, (B) loading plots and (C) VIP results of antioxidant activity of polysaccharides from *N. flagelliforme*.

3.4.2 Structure-radioprotective activity relationship

As shown in Fig. 7A, the samples of WL-EPS-1, NaCl-EPS-1 and Glu-EPS-1 were closely clustered, indicating that there was little difference in radioprotective activity among them. As presented in Fig. 7B, the largest contribution to the radioprotective activity of polysaccharides was attributed to viscosity. The variables with VIP > 1 were viscosity and molecular weight, in descending order (Fig. 7C).

3.4.3 Structure-lipid-lowering activity relationship

As shown in Fig. 8A, the lipid-lowering activity of Glu-EPS-1 and NaCl-EPS-1 had no obvious difference, but was significantly different from that of WL-EPS-1. Fig. 8B shows that viscosity contributed the most to the lipid-lowering activity of polysaccharides. According to the analysis of VIP results, the variables with VIP > 1 were viscosity, molecular weight, total sugar and uronic acid, in descending order (Fig. 8C).

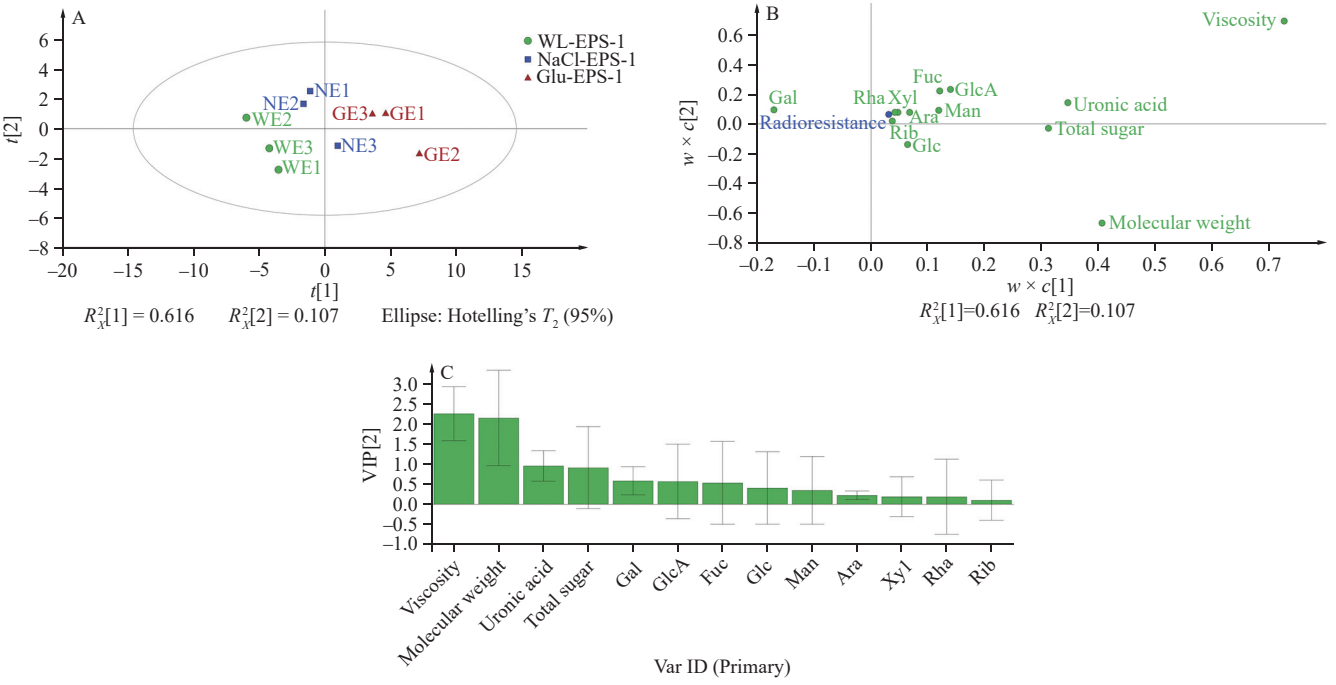

Fig. 7 (A) PLS score plots, (B) loading plots and (C) VIP results of radioprotective activity of polysaccharides from *N. flagelliforme*.

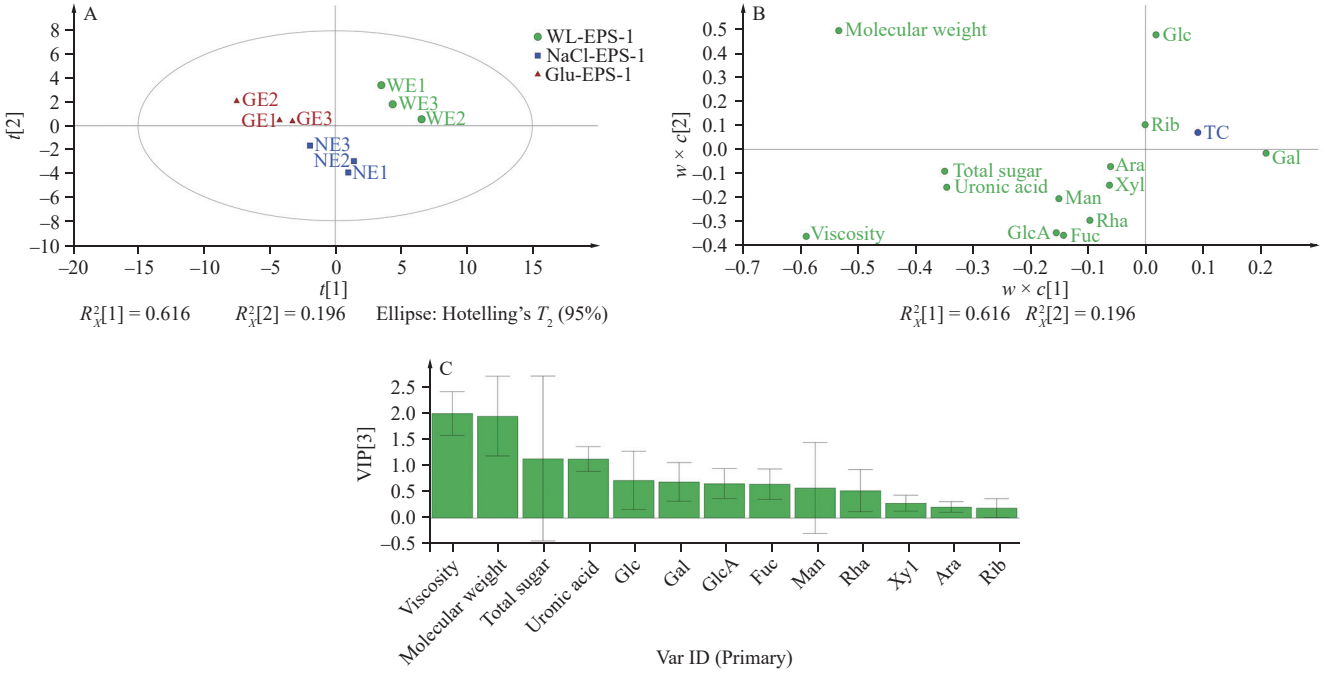

Fig. 8 (A) PLS score plots, (B) loading plots and (C) VIP results of lipid-lowering activity of polysaccharides from *N. flagelliforme*.

3.4.4 Relationship of polysaccharide structure with antioxidant, radioprotective and lipid-lowering activities

As shown in Fig. 9A, the sample points of the three polysaccharides were clearly separated, indicating differences in their antioxidant, radioprotective and lipid-lowering activities. The loading plots showed that viscosity contributed the most to the bioactivity of polysaccharides (Fig. 9B). The VIP results showed that the variables with VIP > 1 were viscosity, molecular weight, uronic acid and total sugar, in descending order (Fig. 9C).

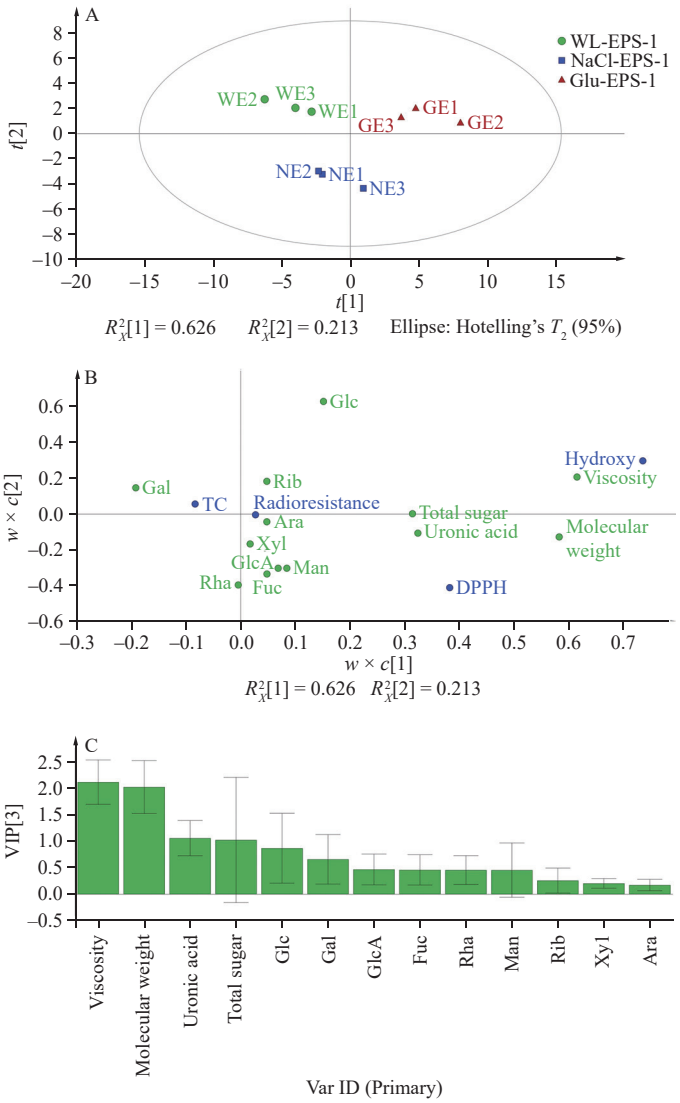

Fig. 9 (A) PLS score plots, (B) loading plots and (C) VIP results of antioxidant, radioprotective and lipid-lowering activity of polysaccharides from *N. flagelliforme*.

Based on the PLS analysis presented above, it was found that the viscosity and molecular weight were the key factors affecting the bioactivity of *N. flagelliforme* polysaccharides. Similarly, Bi[51] found that the monosaccharide composition and glycosidic linkage types of two polysaccharide fractions were basically the same, but the antioxidant activity of BIWS was more significant than that of BIWS-4b, and the molecular weight of BIWS-4b was smaller than that of BIWS. Finally, the authors speculated that a higher molecular weight of the polysaccharide results in higher antioxidant activity. In addition, a large number of studies have reported that polysaccharides with high molecular weight tend to have higher biological activity[30,52-53]. For instance, Chen et al.[52] found that polysaccharides with high molecular weight had a stronger inhibitory effect on S-180 tumor cells than polysaccharides with low molecular weight.

The intrinsic viscosity is also an important factor affecting the bioactivity of polysaccharides. It has been reported that the cholesterol-lowering effect of some polysaccharides may be attributed to their viscosity, as increased viscosity of intestinal supernatant is highly associated with decreased cholesterol in the serum and liver[54], as well as reduced cholesterol absorption[55]. Hu et al.[56] also confirmed that the high viscosity of the polysaccharide from the seeds of *Plantago asiatica* L. (PLP) contributes to its cholesterol reduction ability. The high viscosity characteristics of polysaccharides may have a certain impact on the hydrodynamic limitation, resulting in the effective binding of polysaccharides to bile acids[57], which may disrupt the formation of micelles, reducing the absorption of cholesterol, and subsequently reducing the serum and liver cholesterol levels[58]. In addition, the high viscosity of PLP helps slow the diffusion of Glc and bind Glc, thereby controlling postprandial blood glucose concentrations[57]. Ou et al.[59] as well as Ebihara et al.[60] also confirmed that polysaccharides reduce the diffusion of Glc due to their viscosity, thus keeping the Glc concentration in the small intestine at a low level. It can therefore be concluded that polysaccharides with high viscosity have relatively high hypoglycemic and hypolipidemic activities.

4. Conclusions

In this study, three polysaccharides WL-CPS-1, NaCl-CPS-1 and Glu-CPS-1 were extracted and purified from *N. flagelliforme* grown under normal, salt stress and mixotrophic culture conditions, respectively. Their physicochemical properties, structural characteristics, as well as their antioxidant, radioprotective and lipid-lowering activities and the underlying structure-activity relationships were investigated. The results showed that the culture conditions had significant effects on the proportions of monosaccharides and uronic acid, molecular weight, intrinsic viscosity, triple-helix structure, surface morphology and chain length of the *N. flagelliforme* polysaccharides. *In vitro* bioactivity assays showed that the lipid-lowering activity was consistent with the antioxidant activity, with Glu-CPS-1 showing the highest antioxidant and lipid-lowering activities. However, NaCl-CPS-1 had the strongest radioprotective ability. Moreover, PLS analysis showed that viscosity and molecular weight were the key factors affecting the antioxidant, lipid-lowering and radioprotective activities of polysaccharides. These results demonstrated that the antioxidant, lipid-lowering and radioprotective activities of polysaccharides could be improved by appropriate regulation of culture conditions, which was mainly related to the changes of viscosity, molecular weight, uronic acid content and total sugar content of polysaccharides.

Conflict of interest

There are no conflicts to declare.

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Appendix A. Supplementary information

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSHW.2024.9250292.

References

[1] Q.L. Tang, G. L. Huang, Preparation and antioxidant activities of cuaurbi polysaccharide, Int. J. Biol. Macromol. 117 (2018) 362-365. https://doi.org/10.1016/j.ijbiomac.2018.05.213.

[2] J.L. Wang, A.J. Bao, X.H. Meng, et al., An efficient approach to prepare sulfated polysaccharide and evaluation of anti-tumor activities *in vitro*, Carbohydr. Polym. 184 (2018) 366-375. https://doi.org/10.1016/j.carbpol.2017.12.065.

[3] X.M. Wang, Z.S. Zhang, Z.Y. Yao, et al., Sulfation, anticoagulant and antioxidant activities of polysaccharide from green algae *Enteromorpha linza*, Int. J. Biol. Macromol. 58 (2013) 225-230. https://doi. org/10.1016/j.ijbiomac.2013.04.005.

[4] T. H. Yang, M. Jia, S.Y. Zhou, et al., Antivirus and immune enhancement activities of sulfated polysaccharide from *Angelica sinensis*, Int. J. Biol. Macromol. 50 (2012) 768-772. https://doi.org/10.1016/j.ijbiomac.2011.11.027.

[5] L.M. Zhao, Y.L. Jia, M. Ma, et al., Prevention effects of *Schisandra* polysaccharide on radiation-induced immune system dysfunction, Int. J. Biol. Macromol. 76 (2015) 63-69. https://doi.org/10.1016/j.ijbiomac.2015.02.020.

[6] J.H. Xie, X. Liu, M.Y. Shen, et al., Purification, physicochemical characterisation and anticancer activity of a polysaccharide from *Cyclocarya paliurus* leaves, Food Chem. 136 (2013) 1453-1460. https://doi.org/10.1016/j.foodchem.2012.09.078.

[7] J. Jiang, F.Y. Meng, Z. He, et al., Sulfated modification of longan polysaccharide and its immunomodulatory and antitumor activity *in vitro*, Int. J. Biol. Macromol. 67 (2014) 323-329. https://doi.org/10.1016/j.ijbiomac.2014.03.030.

[8] H.F. Li, Z.W. Li, Y.M. Liu, et al., Prospect for present status and application of polysaccharides from *Nostoc*, J. Agric. Sci. Technol. 13 (2011) 105-110.

[9] H. Fan, Cloning and prokaryotic expression of polysaccharide metabolism-related differently expressed genes from *Nostoc flagelliforme* under salt stress, Xi'an, Shaanxi University of Science and Technology, 2017, pp.3-4.

[10] S.R. Jia, H.F. Yu, Y.X. Lin, et al., Characterization of extracellular polysaccharides from *Nostoc flagelliforme* cells in liquid suspension culture, Biotechnol. Bioprocess Eng. 12 (2007) 271-275. http://doi.org/10.1007/BF02931103.

[11] K.W. Wang, C. Yang, S.N. Yan, et al., *Dendrobium hancockii* polysaccharides, structure characterization, modification, antioxidant and antibacterial activity, Ind. Crops Prod. 188 (2022) 115565. https://doi.org/10.1016/j.indcrop.2022.115565.

[12] M. Lu, X. F. Chen, X.X. Zhao, et al., Effects of salt stress on structural characteristics and functional properties of extracellular polysaccharide from *Nostoc flagelliforme*, Food Ferment. Ind. 42 (2016) 119-124. https://doi.org/10.13995/j.cnki.11-1802/ts.201603021.

[13] G. J. Grobben, M.R. Smith, J. Sikkema, et al., Influence of fructose and glucose on the production of exopolysaccharides and the activities of enzymes involved in the sugar metabolism and the synthesis of sugar nucleotides in *Lactobacillus delbrueckii* subsp. *bulgaricus* NCFB 2772, Appl. Microbiol. Biotechnol. 46 (1996) 279-284. https://doi.org/10.1007/s002530050817.

[14] P.B. Magdalena, C. Adam, W. Adam, et al., Physicochemical characterization of exopolysaccharides produced by *Lactobacillus rhamnosus* on various carbon sources, Carbohydr. Polym. 117 (2015) 501-509. https://doi.org/10.1016/j.carbpol.2014.10.006.

[15] S.G. Shen, S.R. Jia, Y.K. Wu, et al., Effect of culture conditions on the physicochemical properties and antioxidant activities of polysaccharides from *Nostoc flagelliforme*, Carbohydr. Polym. 198 (2018) 426-433. https://doi.org/10.1016/j.carbpol.2018.06.111.

[16] P.P. Han, S. Ying, S.R. Jia, et al., Effects of light wavelengths on extracellular and capsular polysaccharide production by *Nostoc flagelliforme*, Carbohydr. Polym. 105 (2014) 145-151. https://doi.org/10.1016/j.carbpol.2014.01.061.

[17] X. Yang, M. Huang, C. Qin, et al., Structural characterization and evaluation of the antioxidant activities of polysaccharides extracted from qingzhuan brick tea, Int. J. Biol. Macromol. 101 (2017) 768-775. https://doi.org/10.1016/j.ijbiomac.2017.03.189.

[18] L.P. Fan, S.D. Ding, L.Z. Ai, et al., Antitumor and immunomodulatory activity of water-soluble polysaccharide from *Inonotus obliquus*, Carbohydr. Polym. 90 (2012) 870-874. https://doi.org/10.1016/j.carbpol.2012.06.013.

[19] P.P. Han, S.Y. Yao, R.J. Guo, et al., The relationship between monosaccharide composition of extracellular polysaccharide and activities of related enzymes in *Nostoc flagelliforme* under different culture conditions, Carbohydr. Polym. 174 (2017) 111-119. https://doi.org/10.1016/j.carbpol.2017.05.093.

[20] Z.Y. Zhu, F.Y. Dong, X.C. Liu, et al., Effects of extraction methods on the yield, chemical structure and anti-tumor activity of polysaccharides from *Cordyceps gunnii* mycelia, Carbohydr. Polym. 140 (2016) 461-471. https://doi.org/10.1016/j.carbpol.2015.12.053.

[21] Y.R. Li, S.T. Liu, Q. Gan, et al., Four polysaccharides isolated from *Poria cocos* mycelium and fermentation broth supernatant possess different activities on regulating immune response, Int. J. Biol. Macromol. 226 (2023) 935-945. https://doi.org/10.1016/j.ijbiomac.2022.12.077.

[22] Y. Liu, B. Zhang, S. A. Ibrahim, et al., Purification, characterization and antioxidant activity of polysaccharides from *Flammulina velutipes* residue, Carbohydrate Polymers, Carbohydr. Polym. 145 (2016) 71-77. https://doi.org/10.1016/j.carbpol.2016.03.020.

[23] Q.Z. Kang, X.M. Zhang, N.N. Cao, et al., EGCG enhances cancer cells sensitivity under ^{60}Co γ radiation based on miR-34a/Sirt1/p53, Food and Chemical Toxicology, 113 (2019) 110807. https://doi.org/10.1016/j.fct.2019.110807.

[24] X. Li, L. Zhao, Q.H. Zhang, et al., Purification, characterization and bioactivity of polysaccharides from *Glossaulax didyma*, Carbohydr. Polym. 102 (2014) 912-919. https://doi.org/10.1016/j.carbpol.2013.10.057.

[25] X.F. Xie, G.L. Zou, C.H. Li, Purification, characterization and *in vitro* antioxidant activities of polysaccharide from *Chaenomeles speciosa*, Int. J. Biol. Macromol. 92 (2016) 702-707. https://doi.org/10.1016/j.ijbiomac.2016.07.086.

[26] C.Z.P. Nie, P.L. Zhu, S.P. Ma, et al., Purification, characterization and immunomodulatory activity of polysaccharides from stem lettuce, Carbohydr. Polym., 188 (2018) 236-242. https://doi.org/10.1016/j.carbpol.2018.02.009.

[27] M.C. Wang, S.W. Zhao, P. L. Zhu, et al., Purification, characterization and immunomodulatory activity of water extractable polysaccharides from the swollen culms of *Zizania latifolia*, Int. J. Biol. Macromol. 107 (2018) 882-890. https://doi.org/10.1016/j.ijbiomac.2017.09.062.

[28] C.Y. Shen, W.L. Zhang, J.G. Jiang, Immune-enhancing activity of polysaccharides from *Hibiscus sabdariffa* Linn. via MAPK and NF-κB signaling pathways in RAW264.7 cells, J. Funct. Foods 34 (2017) 118-129. https://doi.org/10.1016/j.jff.2017.03.060.

[29] X.L. Ji, F. Liu, N. Ullah, et al., Isolation, purification, and antioxidant activities of polysaccharides from *Ziziphus jujuba* cv. Muzao, Int. J. Food Prop. 21 (2018) 1-11. https://doi.org/10.1080/10942912.2018.1425702.

[30] W.J. Yang, F. Pei, Y. Shi, et al., Purification, characterization and anti-proliferation activity of polysaccharides from *Flammulina velutipes*, Carbohydr. Polym. 88 (2012) 474-480. https://doi.org/10.1016/j.carbpol.2011.12.018.

[31] F.R. Smiderle, L.M. Olsen, A.C. Ruthes, et al., Exopolysaccharides, proteins and lipids in *Pleurotus pulmonarius* submerged culture using different carbon sources, Carbohydr. Polym. 87 (2012) 368-376. https://doi.org/10.1016/j.carbpol.2011.07.063.

[32] Y.J. Zhao, X. Cheng, Effects of carbon sources on fermentation kinetics and structures of exopolysaccharide from *Papiliotrema aureus*, J. Nucl. Agric. Sci. 35 (2021) 384-395. https://doi.org/10.11869/j.issn.100-8551.2021.02.0384.

[33] G.C. Wang, Effects of salt stress on chemical structure and antioxidant activity of *Nostoc Flagelliforme* extracellular Polysaccharide, Shaanxi University of Science and Technology, 2016, pp. 22-51.

[34] H.F. Liu, N.Y. Lin, J. Cheng, et al., Advances in preparation of polysaccharides by microbial fermentation, Food Ferment. Ind. 47 (2021) 281-287. https://doi.org/10.13995/j.cnki.11-1802/ts.026004.

[35] Y.T. Liu, Study on the effect of different liquid fermentation conditions on the active polysaccharides from *Inonotus obliquus*, Northeast Normal University, 2021, pp. 28-51.

[36] C.J. Gao, Y.H. Wang, C.Y. Wang, et al., Antioxidant and immunological activity *in vitro* of polysaccharides from *Gomphidius rutilus* mycelium, Carbohydr. Polym. 92 (2013) 2187-2192. https://doi.org/10.1016/j.carbpol.2012.12.011.

[37] P. Shao, M. Chen, Y.P. Pei, et al., *In intro* antioxidant activities of different sulfated polysaccharides from chlorophytan seaweeds *Ulva fasciata*, Int. J. Biol. Macromol. 59 (2013) 295-300. https://doi.org/10.1016/j.ijbiomac.2013.04.048.

[38] F.O. Diana, C.M. Elizabeth, A.L.E. Jose, et al., Chemical characterization and antioxidant activity of sulfated polysaccharides from *Navicula* sp., Food Hydrocoll. 75 (2018) 229-236. https://doi.org/10.1016/j.foodhyd.2017.08.002.

[39] Z.M. Li, K.Y. Nie, Z.J. Wang, et al., Quantitative structure activity relationship models for the antioxidant activity of polysaccharides, PLoS ONE 11 (2016) e0163536. https://doi.org/10.1371/journal.pone.0163536.

[40] H.Y. Wang, Isolation of UV-B tolerance strain *Pantoea agglomerans* KFS-9 and study on its radioprotective mechanism, Qingdao, Ocean University of China, 2006.

[41] X.J. He, R. Li, G.M. Huang, et al., Influence of marine oligosaccharides on the response of various biological systems to UV irradiation, J. Funct. Foods 5 (2013) 858-868. https://doi.org/10.1016/j.jff.2013.01.035.

[42] S.J. Heo, Y.J. Jeon, Protective effect of fucoxanthin isolated from *Sargassum siliquastrum* on UV-B induced cell damage, J. Photochem. Photobiol., B 95 (2009) 101-107. https://doi.org/10.1016/j.jphotobiol.2008.11.011.

[43] J.L. Zang, Anti-radiation activity and structure analysis of exopolysaccharide produced by *Pantoea agglomerans* KFS, Ocean University of China, 2015, pp. 62-70 .

[44] Z.W. Yang, J. Wang, J.G. Li, et al., Antihyperlipidemic and hepatoprotective activities of polysaccharide fraction from *Cyclocarya paliurus* in high-fat emulsion-induced hyperlipidaemic mice, Carbohydr. Polym. 183 (2018) 11-20. https://doi.org/10.1016/j.carbpol.2017.11.033.

[45] Z.H. Tang, H.W. Gao, S. Wang, et al., Hypolipidemic and antioxidant properties of a polysaccharide fraction from *Enteromorpha prolifera*, Int. J. Biol. Macromol. 58 (2013) 186-189. https://doi.org/10.1016/j.ijbiomac.2013.03.048.

[46] L. Zheng, G. Y. Zhai, J. J. Zhang, et al., Antihyperlipidemic and hepatoprotective activities of mycelia zinc polysaccharide from *Pholiota nameko* SW-02, Int. J. Biol. Macromol. 70 (2014) 523-529. https://doi.org/10.1016/j.ijbiomac.2014.07.037.

[47] L.Q. Wang, N. Xu, J.J. Zhang, et al., Antihyperlipidemic and hepatoprotective activities of residue polysaccharide from *Cordyceps militaris* SU-12, Carbohydr. Polym. 131 (2015) 355-362. https://doi.org/10.1016/j.carbpol.2015.06.016.

[48] X.J. Zhang, Y.H. Hu, C.Z. Jin, et al., Extraction and hypolipidemic activity of low molecular weight polysaccharides isolated from *Rosa Laevigata* fruits, Biomed. Res. Int. 2020 (2020) 2043785. https://doi.org/10.1155/2020/2043785.

[49] F. Naqash, F.A. Masoodi, S. A. Rather, et al., Emerging concepts in the nutraceutical and functional properties of pectin: a review, Carbohydr. Polym. 168 (2017) 227-239. https://doi.org/10.1016/j.carbpol.2017.03.058.

[50] M. Espinal-Ruiz, L.P. Restrepo-Sanchez, C.E. Narvaez-Cuenca, et al., Impact of pectin properties on lipid digestion under simulated gastrointestinal conditions: comparison of citrus and banana passion fruit (*Passiflora tripartite* var. *mollissima*) pectins, Food Hydrocoll. 52 (2016) 329-342. https://doi.org/10.1016/j.foodhyd.2015.05.042.

[51] H.T. Bi, Systematical analysis of the polysaccharides from *Bulgaria inquinans* (Fries) and their biological activities, Changchun, Northeast Normal University, 2007, pp. 32-89.

[52] X.Y. Chen, X.J. Xu, L.N. Zhang, et al., Chain conformation and anti-tumor activities of phosphorylated (1→3)-[*β*]-*D*-glucan from *Poria cocos*, Carbohydr. Polym. 78 (2009) 581-587. https://doi.org/10.1016/j.carbpol.2009.05.019.

[53] Y. Adachi, N. Ohno, M. Ohsawa, et al., Change of biological activities of (1-3)-*β*-*D*-glucan from *Grifola frondosa* upon molecular weight reduction by heat treatment, Chem. Pharm. Bull. 38 (1990) 477-481. https://doi.org/10.1248/cpb.38.477.

[54] D.D. Gallaher, C.A. Hassel, K.J. Lee, Relationships between viscosity of hydroxypropyl methylcellulose and cholesterol in hamsters, J. Nutr. 123 (1993) 1732-1738. https://doi.org/10.1093/jn/123.10.1732.

[55] T.P. Carr, D.D. Gallaher, C.H. Yang, et al., Increased intestinal contents viscosity reduces cholesterol absorption efficiency in hamsters fed hydroxypropyl methylcellulose, J. Nutr. 126 (1996) 1463-1469. https://doi.org/10.1093/jn/126.5.1463.

[56] J.L. Hu, S.P. Nie, C. Li, et al., *In vitro* effects of a novel polysaccharide from the seeds of *Plantago asiatica* L. on intestinal function, Int. J. Biol. Macromol. 54 (2013) 264-269. https://doi.org/10.1016/j.ijbiomac.2012.12.011.

[57] J.Y. Yin, S.P. Nie, J.Li, et al., Mechanism of interactions between calcium and viscous polysaccharide from the seeds of *Plantago asiatica* L., J. Agric. Food Chem. 60 (2012) 7981-7987. https://doi.org/10.1021/jf302052t.

[58] C.M. Gallaher, J. Munion, D.D. Gallaher, et al., Cholesterol reduction by glucomannan and chitosan is mediated by changes in cholesterol absorption and bile acid and fat excretion in rats, J. Nutr. 130 (2000) 2753-2759. https://doi.org/10.1093/jn/130.11.2753.

[59] S. Ou, K. Kwok, Y. Li, et al., *In vitro* study of possible role of dietary fiber in lowering postprandial serum glucose, J. Agric. Food Chem. 49 (2001) 1026-1029. https://doi.org/10.1021/jf000574n.

[60] K. Ebihara, R. Masuhara, S. Kiriyama, Effect on konjac mannan, a water-soluble dietary fiber on plasma glucose and insulin responses in young men undergone glucose tolerance tests, Nutr. Rep. Int. 23 (1981) 577-583.