Carbohydrate Polymers 198 (2018) 426–433

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Effect of culture conditions on the physicochemical properties and antioxidant activities of polysaccharides from *Nostoc flagelliforme*

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ARTICLE INFO

Keywords:
Culture condition
Nostoc flagelliforme polysaccharides
Physicochemical property
Antioxidant activity

ABSTRACT

Three polysaccharides (WL-CPS-1, NaCl-CPS-1 and Glu-CPS-1) were extracted and purified from *Nostoc flagelliforme* under normal, salt stress and mixotrophic culture conditions respectively. Their physicochemical properties and antioxidant activities were investigated. WL-CPS-1, NaCl-CPS-1 and Glu-CPS-1 chemical composition differed in sugar and uronic acid contents, and they were composed of nine constituent monosaccharides and one uronic acid with different ratios, with the average molecular weights of 1.02×10^3, 1.12×10^3 and 1.33×10^3 kDa, respectively. They presented similar fourier transform infrared spectra, but different surface morphology, chain length and branching. Antioxidant assay showed that they all exhibited strong scavenging activity on $ABTS^+$ and hydroxyl radicals and moderate activity on DPPH radical. Glu-CPS-1 exhibited the highest antioxidant activity suggested culture conditions could regulate the bioactivity through influencing the structure and properties. These findings demonstrated the potential application of proper regulation of culture conditions in the development of polysaccharides with high antioxidant activity.

1. Introduction

In recent years, polysaccharides from microalgae have attracted a great deal of attention, because they have a wide range of applications such as antioxidants, antivirus, anti-tumor among others, which has been attributed to their bioactivities and physicochemical properties (Han, Sun, Jia, Zhong, & Tan, 2014; Sun, Wang, Guo, Pu, & Yan, 2014). The bioactivities and physicochemical properties of polysaccharides are determined by their structural features, including molecular weight, monosaccharide composition, conformation and sequence of linkages (Diana et al., 2018; Lo, Chang, Chiu, Tsay, & Jen, 2011; Yan et al., 2017). The microalgal culture conditions are the main factors affecting the structural features, with the stress and nutritional factors as important variables (Han, Yao et al., 2017; Ozturk & Aslim, 2010). In our previous work, it was found that the composition of monosaccharides and the key enzyme controlling polysaccharide synthesis were significantly affected by different culture conditions (Han, Yao et al., 2017). Ozturk and Aslim (2010) also found that the composition of monosaccharides was affected by different salt concentrations. Diana et al. (2018) reported that the antioxidant activity and the average molecular of the polysaccharides synthesized by *Navicula* sp were different under different light conditions. Therefore, it is possible to produce the polysaccharides with high bioactivity by regulating the culture condition.

Oxidation is an essential biological process to many living organisms for the production of energy. Reactive oxygen species (ROS) are constantly formed in the human body and removed by antioxidant defenses, while, excessive amount of ROS can damage cellular components, inducing many diseases including aging and cancer (Chattopadhyay et al., 2010). At present, many synthetic antioxidants are widely used to reduce oxidation damage, but most of them have potential hazards to human health, such as liver damage and carcinogenesis (Fan et al., 2017). Polysaccharides extracted from natural sources are generally acknowledged to have a rather low toxicity leading to their consumption as functional foods (Prajapati, Maheriya, Jani, & Solanki, 2014). Microalgae have been used as a natural source of food for a long time, due their high nutritional content, and they are an important source of bioactive polysaccharides. The antioxidant activity of the polysaccharides from microalgae, such as *Dunaliella* and *Spirulina*, has been extensively described (Alavi & Golmakani, 2017). However, the antioxidant activity of most microalgae is lower than commercial antioxidants, which strongly limits their potential applications in pharmaceutical or nutraceutical fields. Therefore, the strategy of improving the antioxidant activity of the polysaccharides by regulating the culture conditions is proposed.

Nostoc flagelliforme is an edible blue-green algae with great food and

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https://doi.org/10.1016/j.carbpol.2018.06.111
Received 4 February 2018; Received in revised form 10 April 2018; Accepted 26 June 2018
Available online 28 June 2018
0144-8617/

herbal values, which distributes on arid and semi-arid areas. It produces capsular polysaccharide (CPS) strongly bound to the cell surface, which has been reported to possess the properties of antioxidant as well as antivirus, and anti-tumor (Hayashi et al., 2008; Kanekiyo et al., 2005), which makes *N. flagelliforme* as promising resource for the development of functional food. However, considerable research is devoted to increasing the yield of polysaccharides (Ding, Jia, Han, Yuan, & Tan, 2013; Han, Shen et al., 2017), while the quality of the polysaccharides is often ignored. And there is a great lack of knowledge that exists on the relationships between antioxidant activity, structure, and physicochemical property of polysaccharides as well as the factors that regulate *N. flagelliforme* culture. A basic understanding of the relationship of polysaccharide between physicochemical property and antioxidant activity would be helpful for successful application of polysaccharides in functional foods. Therefore, in this study, the effects of different culture conditions including salt stress and mixotrophic culture on the physicochemical properties and antioxidant activities of polysaccharides were investigated. The correlation between physicochemical property and antioxidant activity was subsequently discussed, which aimed to obtain the optimal culture conditions for the production of *N. flagelliforme* polysaccharides with high antioxidant activity in the future.

2. Materials and methods

2.1. Materials and reagents

The *N. flagelliforme* cells (TCCC11757) utilized in liquid suspension cultures were provided by the Tianjin Key Lab of Industrial Microbiology (Tianjin, China).

The standard monosaccharides including glucose, mannose, xylose, rhamnose, arabinose, galactose, fructose, ribose, fucose and glucuronic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade. The ultra-pure water was utilized from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Experimental design

The *N. flagelliforme* cells pre-cultured in 500 mL Erlenmeyer flasks containing 200 mL BG-11 medium under continuous illumination of 60 μmol photons m^{-2} s^{-1} at 25 °C for 10 days were used as inoculums. It was then inoculated (10%, v/v) to a 80 L airlift photo-bioreactor with 60 L BG-11 medium. Then the cultivation of *N. flagelliforme* cells was conducted at 25 °C and 8.0 of pH for 24 days. The aeration rate was set to 0.8 vvm, which had been determined previously to be appropriate for *N. flagelliforme* cell growth (Liu & Chen, 2003). Continuous illumination was provided using 8 fluorescent lamps set into the bioreactor, generating an average light intensity of 60 μmol photons m^{-2} s^{-1}. The experiments were consists of three groups: control group, salt stress group and mixotrophic culture group.

Control group: cells were cultured in BG-11 medium for 24 days.

Salt stress group: cells were first cultured in BG-11 medium for 15 days, then NaCl was added to a final concentration of 0.5 mol/L, and continued for culturing 9 days.

Mixotrophic culture group: cells were first cultured in BG-11 medium for 15 days, then glucose was added to a final concentration of 4 g/L, and continued for culturing 9 days.

2.3. Preparation and purification of polysaccharides

The *N. flagelliforme* culture was centrifuged at 10,000 × g for 10 min at 4 °C to harvest cells, which would be further processed for extraction of polysaccharides. The crude polysaccharides were obtained by extracting lyophilized cell in hot water at 80 °C for 6 h, then concentrating by ultra-filtration and finally freeze-drying. The crude polysaccharides would then be redissolved in ultra-pure water and further purified according to the previously described (Han et al., 2014).

2.4. Yield of crude polysaccharide

The total polysaccharides were detected by phenol-sulfuric acid assay (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

$$\text{Yield of crude polysaccharide (\%)} = W_1/W_2 \times 100\% \quad (1)$$

Where W_1 is the content of polysaccharide and W_2 is the weight of N. flagelliforme powder.

2.5. Chemical analysis

Total sugar contents were determined by phenol-sulfuric acid method (Dubois et al., 1956). The protein contents were determined according to Bradford's method (Zhu et al., 2017). The uronic acid contents were determined using sulfuric acid carbazole colorimetry method (Fan, Ding, Ai, & Deng, 2012). The total phenol contents were assayed by Folin-Ciocalteu's colorimetry (Yang et al., 2017).

The monosaccharide compositions of polysaccharides were analyzed according to the method previously reported (Han, Yao et al., 2017).

The homogeneity and molecular weight (*MW*) of polysaccharides were determined by High Performance Size Exclusion Chromatography (HPSEC) on TSK-Gel G4000 PWXL (30 cm × 7.5 mm) with a refractive index detector. The mobile phase was ultra-pure water with a flow rate of 0.5 mL/min. The injection volume was 20 μL, and the column and detector temperature were 35 °C. Dextran standards of various molecular weights were used to establish a standard curve. The molecular weight of polysaccharides was estimated according to the retention time and Log *MW* of the dextran standards.

2.6. Structural characterization

2.6.1. Ultraviolet (UV) spectra

Each polysaccharide sample solution (1 mg/mL) was analyzed by UV spectroscopy on a UV-2401 ultraviolet spectrophotometer (SHIMADZU Corporation, Japan) at wavelengths in the range of 200–400 nm.

2.6.2. Fourier-transform infrared (FT-IR) spectra

Each polysaccharide sample was analyzed by FT-IR spectroscopy on a VECTOR 22 Fourier transform infrared spectrometer (Bruker Corporation, Germany). Dried samples were used for the FT-IR measurements and ground with KBr power before being pressed into pellets for analysis at wave numbers from 4000 to 400 cm^{-1}.

2.6.3. Triple helical structure analysis

The triple helical structures of polysaccharides were analyzed by their interaction with Congo red. 1 mL of polysaccharide solution (1 mg/mL) was mixed with 1.0 mL of 80 μmol/L of Congo red in a gradient of NaOH solutions (0, 0.1, 0.2, 0.3, 0.4 and 0.5 mol/L). The absorbance was measured in the range of 400–600 nm, and the maximum absorption wavelength at different concentrations of NaOH was plotted. Distilled water without addition of polysaccharide was measured as the control.

2.6.4. Molecular surface morphology analysis

Scanning electron microscope (SEM) (FEI Company, The Netherlands) was used to observe the morphology of the polysaccharide sample. The sample coating with a thin gold layer was placed on the substrate. The polysaccharide sample was then observed at the voltage of 5.0 kV.

Atomic force microscope (AFM) was used in tapping mode to obtain the topographies of the sample. Briefly, the sample was dissolved in ultra-pure water to get a final concentration of 5 μg/mL. Subsequently,