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Effects of light wavelengths on extracellular and capsular polysaccharide production by *Nostoc flagelliforme*

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ABSTRACT

The influences of different wavelengths of light (red 660 nm, yellow 590 nm, green 520 nm, blue 460 nm, purple 400 nm) and white light on extracellular polysaccharide (EPS) and capsular polysaccharide (CPS) production by *Nostoc flagelliforme* in liquid culture were demonstrated in this study. The results showed that, compared with white light, red and blue lights significantly increased both EPS and CPS production while yellow light reduced their production; purple and green lights stimulated EPS production but inhibited CPS formation. Nine constituent monosaccharides and one uronic acid were detected in both EPS and CPS, and their ratios showed significant differences among treatment with different light wavelengths. However, the advanced structure of EPS and CPS from various light conditions did not present obvious difference through Fourier transform infrared spectroscopy and X-ray diffraction characterization. These findings establish a basis for development of high-yielding polysaccharide production process and understanding their regulation.

1. Introduction

Natural polysaccharides are biologically produced polymeric materials, and organized in two distinct morphological forms: (1) as capsules, strongly bound to the external cell surface (CPS fraction) and (2) as slimy polysaccharides, either loosely attached to the capsules, or released into the surrounding environment (EPS fraction) (Rehm, 2010). Interest in polysaccharides derived from cyanobacteria has increased significantly in recent years, as these biopolymers often evidence advantages over other polysaccharides extracted from plants or marine microalgae (Sutherland, 1998). Due to the wide applications in the area such as improvement of water-holding capacity of soil, removal of heavy metals from wastewater, and being used as food additives (Bender & Phillips, 2004; Freire-Nordi, Vieira, & Nascimento, 2005), the demand of cyanobacterial polysaccharide as newly emerging industrially important biopolymers has kept increasing.

Nostoc flagelliforme is an edible terrestrial cyanobacteria with great economic value, which is distributed throughout arid and semi-arid areas. The EPS of *N. flagelliforme* has been proved to possess the properties of antivirus, antioxidant, and antitumor (Jia et al., 2008; Kanekiyo et al., 2005); besides that, it has been reported with high intrinsic viscosity, good emulsification activity, and excellent flocculation capability, and considered as a very promising candidate for numerous industrial applications (Han et al., 2013). While the low yield of EPS plus the lack of information regarding the factors controlling its biosynthetic processes, strongly limits their potential for biotechnological applications.

Cyanobacteria are a group of photoautotrophic microorganisms that have both a photosynthetic apparatus that converts light into chemical energy and photoreceptor proteins that sense the wavelength and intensity of light and then transduce this information into various cellular pathways. The control and optimization of light wavelength and intensity is regarded as one of the most important parameters for the culture of photosynthetic microorganisms (Ugwu, Aoyagi, & Uchiyama, 2008). Additionally, it has been reported that continuous light and high light intensities could enhance EPS production (Otero & Vincenzini, 2003; Trabelsi, Ouada, Bacha, & Ghoul, 2009). And certain light wavelengths have also been demonstrated to influence EPS production; notably, in the heterocystous *Nosotc commune*, UV-B irradiation stimulates extracellular glycan production (Ehling-Schulz, Bilger, & Scherer, 1997). Those results indicated that the synthesis and release of cyanobacterial polysaccharide might be particularly light dependent. Yet the

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comprehensive study of the effects of light on the production, structure, and characteristics of cyanobacterial polysaccharide is still lacking.

No information is available on the effects of light wavelengths on the polysaccharide production of *N. flagelliforme*. Therefore, in this study, the influences of light wavelengths on production, composition, and structure of both EPS and CPS were comprehensively investigated using red, yellow, green, blue, purple light emitting diodes for illumination, in order to establish the key culture factors driving to the maximization of polysaccharide production. Our results showed that red and blue lights were more effective for production of EPS and CPS than other monochromatic lights and white light, respectively.

2. Materials and methods

2.1. Strain and culture conditions

The *N. flagelliforme* cells (TCCC11757) utilized in liquid suspension cultures were obtained from the Tianjin Key Lab of Industrial Microbiology (Tianjin, China). The cells were cultured in BG-11 medium in 1000 mL shake-flask containing 500 mL medium at 25 °C under continuous illumination at a photo flux density of 60 μmol photon/(m^2 s) for monochromatic red (660 nm), yellow (590 nm), green (520 nm), blue (460 nm), and purple (400 nm). The half-band widths are 5 nm for each monochromatic light, according to manufacturer's instruction (Shenzhen federal heavy secco electronic Co. LTD., China). The cells grown under white light were treated as control.

2.2. The measurement of cell growth, EPS production, and CPS production

The cell growth was measured by weighing the cell dry weight. The polysaccharide production was determined via phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956).

2.3. Extraction and purification of EPS and CPS

The *N. flagelliforme* culture was centrifuged at 15,000 × *g* for 20 min at 4 °C to harvest supernatant and cells separately, which would be further processed for extraction of EPS and CPS respectively. The supernatant was concentrated using ultra-filtration equipment (UEIP-503 Motianmo Group of Tianjin Polytechnic University, China) and then freeze dried to get crude EPS. The crude CPS was 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 EPS and CPS would then be redissolved in deionized water respectively and further purified according to the following procedure. The solution was then dealt with DEAE-52 cellulose anion exchange column (2.6 cm × 60 cm) (TOSOH Corporation, Japan). The column was eluted with deionized water first, and then with a linear gradient of 0–1.0 mol/L NaCl solution at a flow rate of 1.0 mL/min. The fractions were collected and the carbohydrates were monitored via phenol-sulfuric acid method (Dubois et al., 1956). Carbohydrate-positive fractions were merged, lyophilized and then processed with a Sephadex G100 gel chromatographic column (Φ1.6 cm × 80 cm, Pharmacia Corporation, Sweden). The column was eluted with 300 mL of distilled water at a flow rate of 0.5 mL/min. The fractions were collected and the carbohydrates were monitored via phenol-sulfuric acid method. Carbohydrate-positive fractions were merged and lyophilized to get the purified product.

2.4. Determination of monosaccharide compositions of polysaccharide

The monosaccharide compositions of the EPS and CPS were determined by gas chromatography–mass spectrometry (GC–MS, Agilent Technologies, CA, USA) of their trimethylsilyl (TMS) derivatives obtained after acidic hydrolysis and derivatization as previously described (Han et al., 2013).

2.5. Fourier transformed infrared (FT-IR) spectroscopy

The IR spectra were characterized using a VECTOR 22 Fourier transform infrared spectrometer (Bruker Corporation, Germany). The polysaccharide was freeze-dried, mixed with KBr powder and pressed into pellets, then scanned at wave numbers from 4000 to 400 cm^{-1}.

2.6. X-ray diffraction (XRD)

X-ray measurements were carried out to analyze the changes in crystallinity of polysaccharide using a Bruker D8 Advance X-ray diffractometer (Bruker-AXS Corp, Germany) with reflection geometry and CuKα radiation (λ = 0.154 nm), operated at 40 kV, and 30 mA. The scanning was made through 2θ = 0–80° with a step interval of 0.02°. MDI Jade 5.0 was used to process the diffraction pattern and calculate the crystallinity of the sample (Lu, Das, Li, Peng, & Zhang, 2013).

3. Results and discussion

3.1. Biomass accumulation

After pre-cultivating the cells under fluorescent light, they were incubated in the dark for 3 days to reduce storage compounds and avoid the influence of light from pre-culture. Then, cells were transferred to continuous illumination with monochromatic light of 60 μmol photon/(m^2 s). *N. flagelliforme* was sensitive to the changes of light spectrum, and a significant difference in cell growth was observed in Fig. 1. The biomass was higher in the order of red, blue, green, white, yellow, and purple-grown cells with values of 0.70, 0.68, 0.63, 0.62, 0.58, 0.56 g/L, respectively, after 15 days of culture (Fig. 1A). The maximum average growth rate (Fig. 1B) was 0.42 d^{-1} and achieved by cells grown at red light, which was followed by blue, white, green, yellow, and purple lights. Red and blue lights promoted rapider growth than white light. Green light had similar effect with white light. While, yellow and purple lights reduced cell growth rate compared with white light. The results indicated that red and blue lights were more suitable for cell growth and promoted cells with higher growth rates.

3.2. EPS and CPS production

The time courses of EPS production under different light conditions were presented in Fig. 2. After 15 days of culture, EPS production was more in the order of blue, red, purple, green, white, and yellow-grown cells, which reached 32.1, 30.9, 23.9, 21.6, 19.4, 17.2 mg/L respectively. The EPS production of blue-light and red-light grown cells were 1.65 and 1.59 times to that of white-light grown cells. Purple light and green light increased EPS production, but to a lesser extent than blue and red lights. Yellow light reduced EPS production compared with white light.

To compare the polysaccharide-producing capacities of *N. flagelliforme* cells under different light conditions, the EPS and CPS production per unit cell mass was calculated and listed in Table 1. Blue-light grown cells possessed highest EPS productivity of 47.39 mg/g DCW, followed by red-, purple-, green-, white-, and