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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

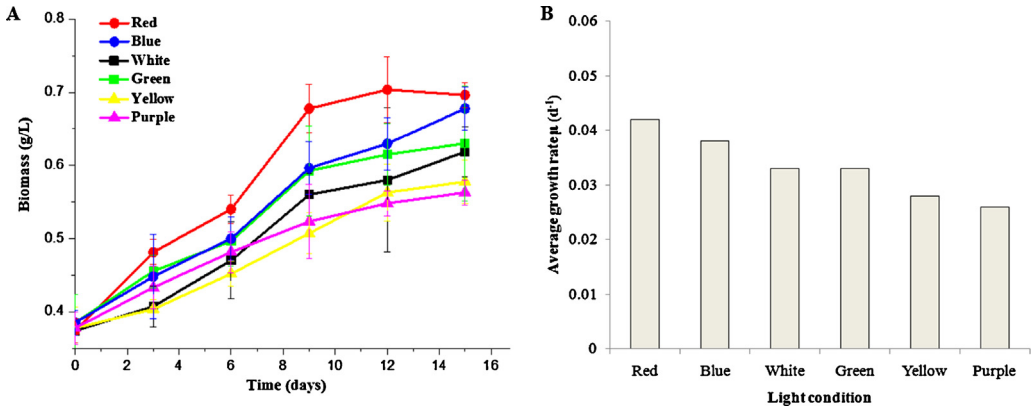

Fig. 1. The effects of light with different wavelengths on the growth of *Nostoc flagelliforme* cells. (A) The growth curves. (B) The average cell growth rates. Data represent means ± SD of 3 independent experiments.

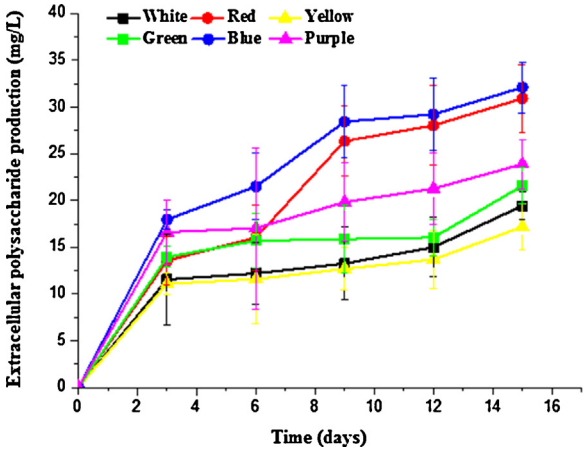

Fig. 2. The effects of light with different wavelengths on the extracellular polysaccharide production of *Nostoc flagelliforme* cells. Data represent means ± SD of 3 independent experiments.

yellow-light grown cells with EPS productivities of 44.44, 42.42, 34.24, 31.43, and 29.72 mg/g DCW, respectively. For CPS productivity, the values of red- and blue-light grown cells were 261.37 and 229.78 mg/g DCW, which were 1.43 and 1.26 times to that of white light-grown cells, respectively. Purple-light grown cells produced the least amount of CPS, the productivity of which was 61% of that of white-light grown cells. From the above results, the maximum production of EPS and CPS was achieved by blue- and red-light conditions respectively, indicating light wavelengths have important effects on both capsular and extracellular polysaccharide production, as found in other studies with different algae species (Carmona, Vergara, Lahaye, & Niell, 1998; Singh & Das, 2011; You & Barnett, 2004). However, the influence of the same monochromatic light on polysaccharide production was quite different among various strains, which suggested that the effects of light wavelengths on polysaccharide production might vary depending on the strains.

Table 1
The effects of light wavelengths on polysaccharide production of *Nostoc flagerlliforme* cells after 15 days of culture.

Light condition	EPS production (mg/g DCW)	CPS production (mg/g DCW)	Ratio of EPS to CPS
White	31.43 ± 0.71	182.94 ± 12.91	17%
Red	44.44 ± 3.63	261.37 ± 20.11	17%
Yellow	29.72 ± 2.43	125.64 ± 7.93	24%
Green	34.24 ± 2.01	164.21 ± 9.24	21%
Blue	47.39 ± 2.77	229.78 ± 12.32	21%
Purple	42.42 ± 2.70	112.22 ± 7.41	38%

The ratio of EPS to CPS has been calculated to indicate the extent of polysaccharides release into the extracellular environment. From Table 1, it was shown that the highest ratio of EPS to CPS was 38% occurring in the purple-grown cells, which was followed by yellow-, green-, blue-, white-, and red-light grown cells. When cells grow under white and red lights, the ratios of EPS to CPS were 17% and lower than that of other light conditions, referring that cells do not tend to release EPS under white and red light conditions.

From Fig. 1 and Table 1, it could be inferred that CPS production and cell growth were positively correlated. The cells with higher growth rate could produce more polysaccharide. Results of these experiments indicated a dependence of the growth rate and polysaccharides production on the energy of radiation and spectral distribution of light. While the biosynthesis of EPS was more closely related with culture conditions compared with CPS production. Purple- and yellow-light grown cells with lower growth rates possessed higher ratios of EPS to CPS than other light grown cells. These results confirm that the kinetics of EPS release may not be associated with the cell growth dynamics, but more dependent on the culture conditions. This corresponds with the findings of many reports (De Philippis, Margheri, Materassi, & Vincenzini, 1998; Mouhim, Cornet, Fontaine, Fournet, & Dubertret, 1993; Trabelsi et al., 2009).

3.3. Monosaccharide compositions

The monosaccharide composition was analyzed by GC–MS after acid hydrolysis and subsequent trimethylsilyl derivatization. After complete acidic hydrolysis of obtained EPSs and CPSs, nine constituent monosaccharides glucose, galactose, mannose, ribose, xylose, arabinose, rhamnose, fucose, fructose plus one uronic acid glucuronic acid were detected in different combinations and ratios. The most abundant monosaccharide in EPS (Fig. 3) of *N. flagelliforme* cells under all tested light conditions in this study was glucose, the ratio of which ranged from 35.71% to 43.08%. There were no significant differences in the ratios of galactose among white-, red-, blue-, green-, and purple-light grown cells; but the ratio in yellow-light grown cells was obviously lower than that of other light-grown cells. The ratios of mannose in purple- and yellow-light grown cells were both around 15% and lower than that of other light grown cells. The differences in ratios of fructose between red-light grown cells and yellow-light grown ones were quite significant; the ratio of the former was 0.74% but in the latter was 32.04%, while in other light grown cells it was in the range of 7–14%. The highest ratio of glucuronic acid was 6.24% and occurred in the purple-light grown cells, followed by green-, blue-, red-, white-, and yellow-light grown cells, respectively. The EPS of red-light grown cells had

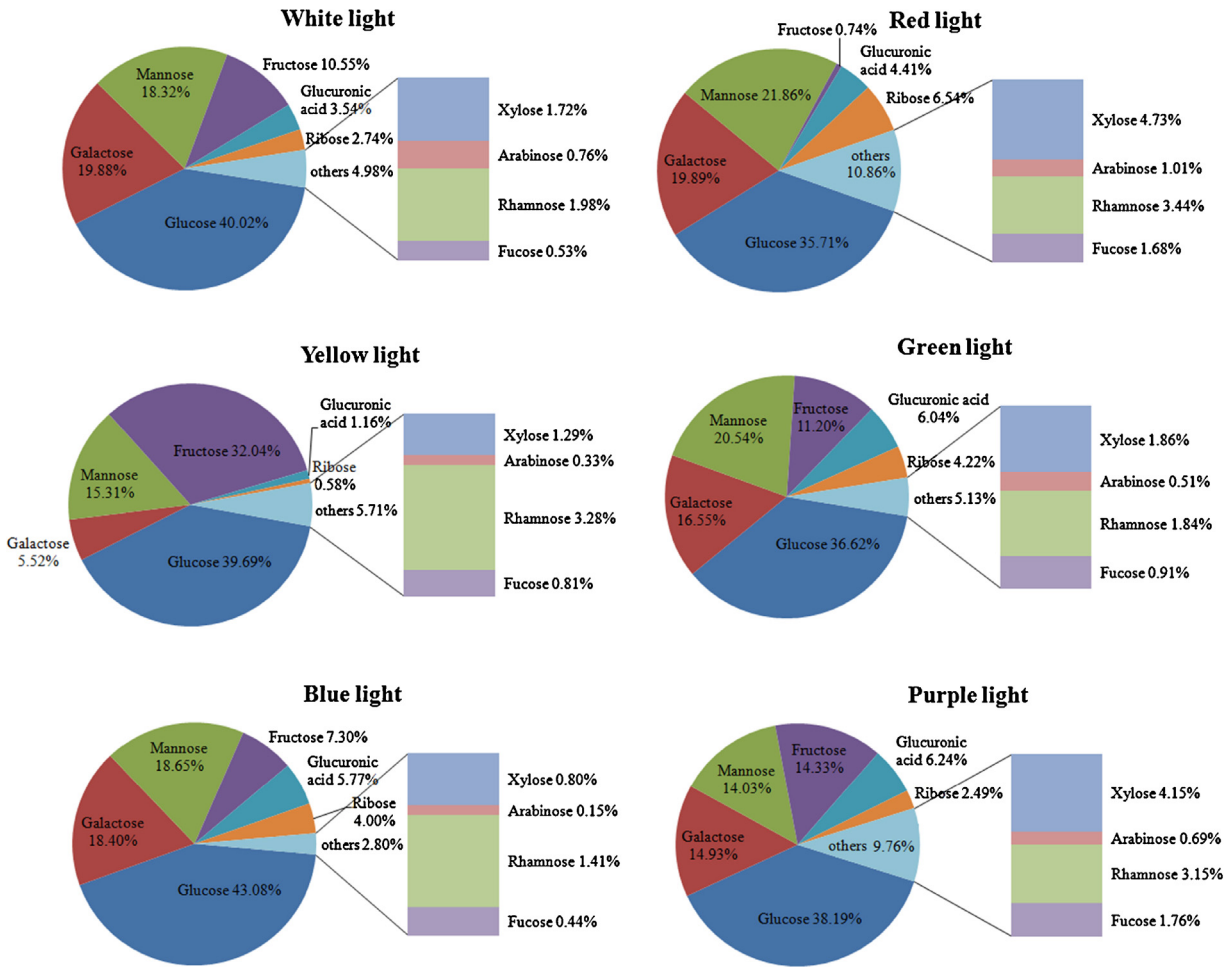

Fig. 3. The effects of light with different wavelengths on the monosaccharide compositions of extracellular polysaccharide produced by *Nostoc flagelliforme* cells.

the highest content of ribose with 6.54%, while EPS of yellow-light grown cells contained lowest amount of 0.58%. The proportion of the rest four monosaccharides xylose, arabinose, rhamnose, and fucose in EPS was quite small compared with other monosaccharides; the highest ratio of the sum of them was observed in red-light grown cells and just reached 10.86%.

From the results of CPS monosaccharide composition in different light grown cells presented in Fig. 4, glucose remained the most abundant one and its contents in CPS from different light grown cells were very close and in the range of 46.58–54.68%, while the ratios of glucose of CPS under all tested light conditions in this study were apparently higher than that of EPS. No significant differences in the ratios of galactose of CPS among white-, red-, blue-, green-, yellow-, and purple-light grown cells was observed, however, the ratios of galactose in CPS had overall higher elevations compared with that of EPS except the red-light grown cells with the same proportion of galactose in EPS and CPS. The content of mannose in CPS of white-light grown cells was significantly lower than that of other light grown cells; in addition, the ratios of mannose in CPS of all light conditions were obviously less than that in EPS. For fructose as a monomer in CPS, among all light conditions, green-light grown cells had the smallest ratio at 0.35%, and red-light grown cells were with the largest ratio at 15.63%. The proportion of fructose in CPS of yellow-light grown cells was 4.06% and lower than that of red-light grown cells, which was opposite to the results of EPS composition presented in Fig. 3 in the above mentioned light conditions. The highest ratio of glucuronic acid in CPS was 7.80% and occurred in the white-light grown cells. There were not dramatic differences observed in the ratios of ribose in CPS from different light grown cells. The sum of proportions of the rest four monosaccharides xylose, arabinose, rhamnose, and fucose in CPS was in the range of 1.6–3.6%, and overally lower than that in EPS.

The influence of light wavelengths on monosaccharide composition of both EPS and CPS was significant, indicative of the effects of light wavelengths on polysaccharide biosynthetic pathways. The biosynthesis and secretion of polysaccharide in cyanobacteria probably follow the pathways conserved throughout other bacteria; however, as a consequence of the cyanobacteria's ability to perform oxygenic photosynthesis, the difference of the production of cyanobacterial exopolysaccharides is intimately dependent on the balance between the catabolic pathways of sugar degradation and the anabolic pathways of sugar nucleotide synthesis (Pereira et al., 2009). There were literatures demonstrating that carbon metabolism in some photosynthetic organisms such as *Chlorella* and land plants, was regulated by a particular wavelength of light (Kami, Lorrain, Hornitschek, & Fankhauser, 2010; Kamiya & Saitoh, 2002). Thus, it might be inferred that the influence of light wavelengths on polysaccharide biosynthesis was exerted via the reprogrammed carbon metabolism depending on the energy availability under different light conditions, which thereby led to the differences in monosaccharide composition.

3.4. FT-IR analysis

The FT-IR spectrums of EPS and CPS from different light conditions were presented in Fig. 5A and B respectively, which displayed

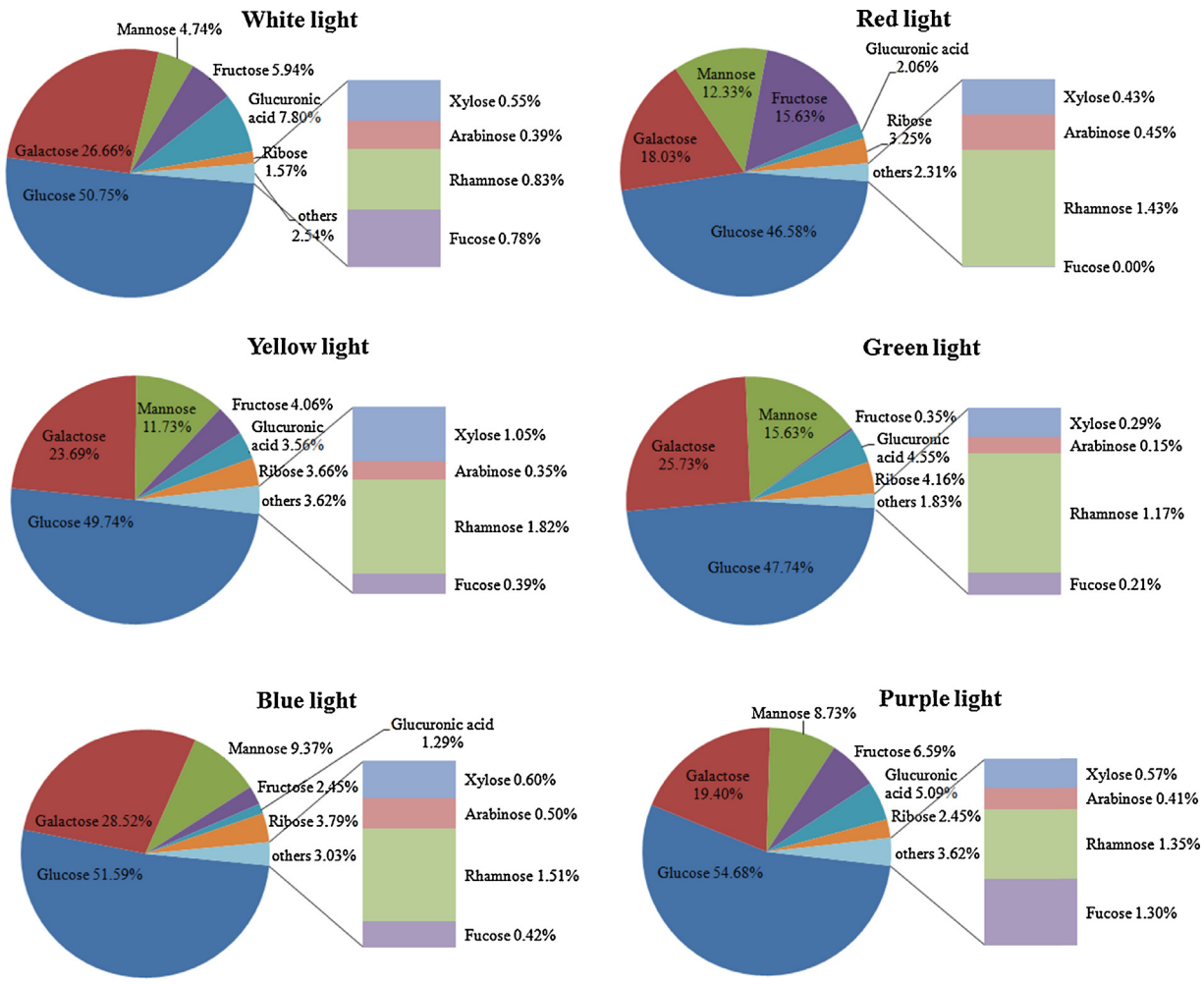

Fig. 4. The effects of light with different wavelengths on the monosaccharide compositions of capsular polysaccharide produced by *Nostoc flagelliforme* cells.

similar characteristics for polysaccharides produced by different light-grown cells. The broad and intense band around 3408 cm^{-1}, common to all polysaccharides, represented O—H stretching of hydroxyl groups (Synytsya, Copikova, Matejka, & Machovic, 2003), which overlapped in part with the C—H stretching peak appearing around 2940 cm^{-1}. The absorption observed at 1658 cm^{-1} was attributed to the C=O stretching vibration of the carboxyl groups. The two bands around 1658 and 1434 cm^{-1} were attributed to the

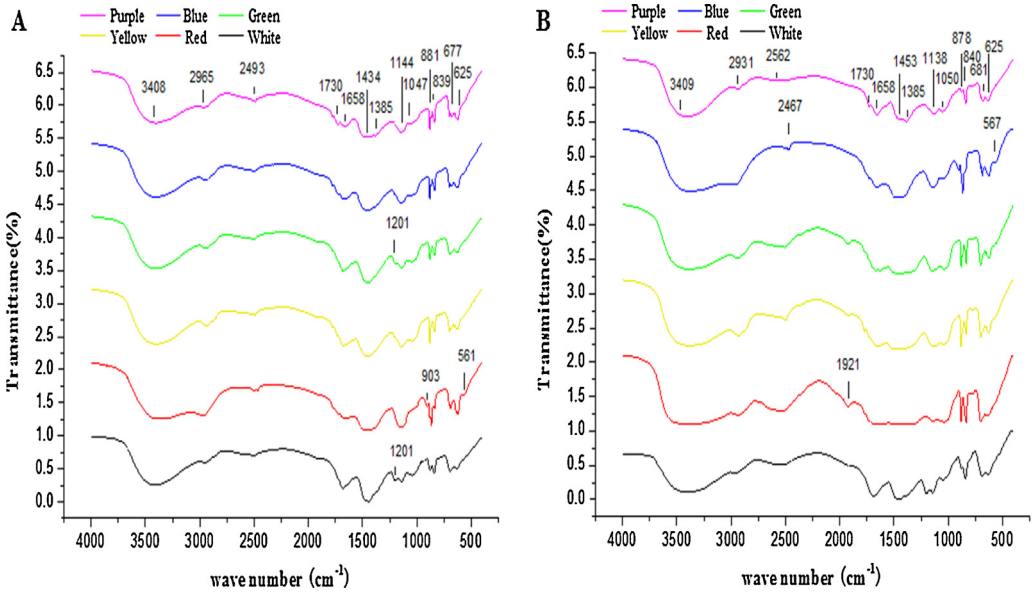

Fig. 5. Comparative FT-IR spectra of extracellular polysaccharide (A) and capsular polysaccharide (B) from *Nostoc flagelliforme* under different light wavelengths.

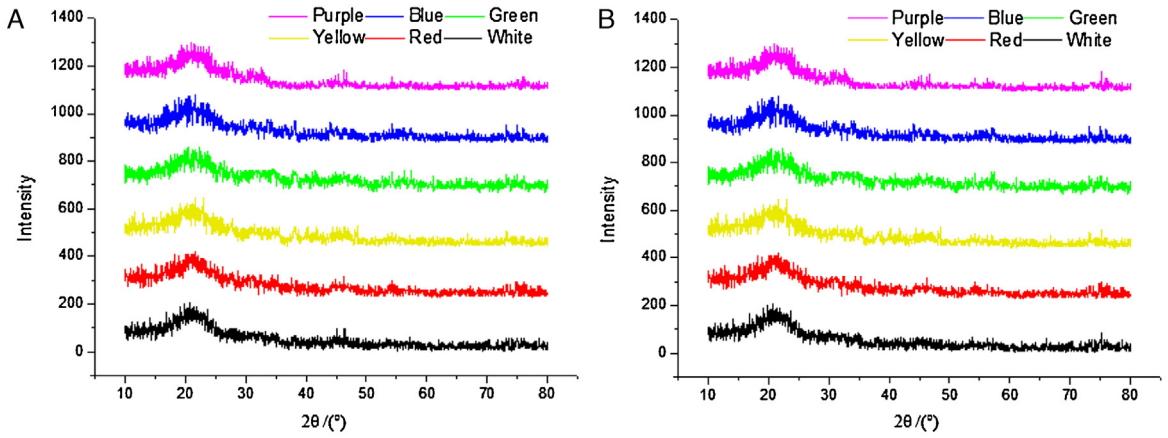

Fig. 6. Comparative XRD analysis of extracellular polysaccharide (A) and capsular polysaccharide (B) from *Nostoc flagelliforme* under different light wavelengths.

stretching vibration of carboxyl groups, which was in agreement with the existence of glucuronic acid in the polysaccharide. The well-defined envelope found between 1200 and 900 cm^{-1} represented skeletal C—O and C—C vibration bands of glycosidic bonds and pyranoid ring. The absorption around 880 cm^{-1} indicated EPS and CPS had β-type glycosidic linkages. Compared to the great differences in monosaccharide composition observed in polysaccharides from different light grown cells, no relevant shifting of peaks has been found with FT-IR spectra, although the different monosaccharide composition might affect the absorption intensity in the spectra.

3.5. XRD analysis

The X-ray diffraction patterns were obtained to compare the microstructural changes in polysaccharides from different light conditions. As presented in Fig. 6, X-ray diffractograms of both EPS and CPS presented "bun-shaped" curves which suggested that the EPS and CPS were noncrystalline. Additionally, no obvious difference has been detected among different light grown cells.

In contrast with differences in monosaccharide composition of EPS and CPS produced by cells grown under different light wavelengths, no significant changes were observed in advanced structure characterized by FT-IR and XRD. The phenomenon that great differences in monosaccharide composition in contrast with no relevant changes in advanced structure of polysaccharide has also been found in the study of EPSs isolated from *Dunaliella salina* under salt stress (Mishra & Jha, 2009). Based on the above mentioned phenomenon, the following might be deduced. Although the biosynthesis and assembly of polysaccharides is a complex process, the regulation mechanism has great flexibility on dealing with changes in a range of environmental conditions, thus the presented advanced structural features of polysaccharide would not be affected even when the monosaccharide composition i.e. repeating unit changed. However, further investigation is required to elucidate the regulation mechanism. Additionally, the biological activities of polysaccharides were closely related to their structures. The structures of polysaccharides such as the main chain, substitution groups, molecular weight and polymerization degree have been reported to affect function of polysaccharides on the activities of anti-tumor, antivirus, and antioxidant, etc. (Ghosh et al., 2009). The differences in functions of polysaccharides producing from different light conditions would require further investigation. The results in this study would provide basic data for further study on the structure-function relationship of polysaccharide from different culture conditions.

4. Conclusions

In this study, EPSs and CPSs from *N. flagelliforme* grown under light with different wavelengths were isolated. The results of this study revealed that blue light and red light were more suitable than white light for growing *N. flagelliforme* cells for the production of EPS and CPS respectively. The monosaccharide compositions of both EPS and CPS showed obvious differences with the changes of light wavelengths. However, the advanced structure of EPS and CPS from various light wavelengths did not present relevant difference through FT-IR and XRD characterization. These findings would establish a basis for development of the process for maximization of polysaccharide production. And further work is required to elucidate the regulation mechanism of light wavelengths on polysaccharide biosynthesis.

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