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Comparative metabolomic analysis of the effects of light quality on polysaccharide production of cyanobacterium *Nostoc flagelliforme*

Pei-pei Han [a,b], Shi-gang Shen [a], Hui-Yan Wang [a], Ying Sun [a], Yu-jie Dai [a], Shi-ru Jia [a,*]

[a] Key Laboratory of Industrial Fermentation Microbiology, Ministry of Education, School of Biotechnology, Tianjin University of Science and Technology, Tianjin 300457, PR China
[b] Key Laboratory of Systems Bioengineering, Ministry of Education, Tianjin University, Tianjin 300072, PR China

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ABSTRACT

Light quality strongly affects cell growth and polysaccharide production of cyanobacteria, however, the metabolic mechanism of polysaccharide biosynthesis upon light quality has not been fully explored yet. The effects of light quality on the metabolic changes of *Nostoc flagelliforme* were comprehensively investigated using red, yellow, green, blue, purple light emitting diodes for illumination. The principal component analysis revealed that red, yellow, green, blue, and purple light-grown cells were metabolically distinct from those illuminated with white fluorescent light, and the influence of light quality on metabolism was particularly dependent on light wavelength. Significant correlations between the metabolic profile and extracellular polysaccharide (EPS) and capsular polysaccharide (CPS) production were revealed by partial least-squares to latent structure analysis respectively, and analysis of key metabolites showed that CPS production was closely related with cell growth while the biosynthesis of EPS was more closely related with light conditions. Furthermore, as the influence pattern of red light on cellular metabolism and polysaccharide production was quite different from other light conditions, the effects of intensity of red light on photosynthesis and polysaccharide production were evaluated, and the results revealed that red light induced photoinhibition and therefore stimulated polysaccharide production. Manipulation of red light was thus proposed to profitably enhance polysaccharide productivity of *N. flagelliforme*.

1. Introduction

As polysaccharides derived from cyanobacteria often evidence advantages over other polysaccharides extracted from plants or marine microalgae, interest in these biopolymers has increased significantly in recent years [1–3]. 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 [4–7], 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 [8,9]. The extracellular polysaccharide (EPS) of *N. flagelliforme* has been proved to possess the properties of antivirus, antioxidant, and anti-tumor [10–13], 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 [14]. *N. flagelliforme* also produces capsular polysaccharide (CPS) strongly bound to the external cell surface, which has been found with similar biological activities with EPS [12,13], which makes *N. flagelliforme* as promising resource for the development of health food. However, the low yield of polysaccharide plus the lack of information regarding the factors controlling its biosynthetic processes, strongly limits their potential for biotechnological applications.

Light is the only source for the generation of energy and reduced carbon (C) for cyanobacteria under photoautotrophic conditions [15,16]. The control and optimization of light wavelength and intensity are regarded as one of the most important parameters for the culture of photosynthetic microorganisms [16–18]. Changes in the spectral composition and intensity of light have been found with significant influence on polysaccharide production. Continuous light and high light intensities could enhance EPS production [19,20]. And certain light wavelengths have also been demonstrated to influence EPS production [21–23], notably, in the heterocystous *Nostoc commune*, UV-B irradiation stimulates extracellular glycan production [24]. We have systematically studied the influences of light wavelengths on production, composition, and structure of both EPS and CPS, and revealed that light wavelengths had significant impact on the product and monosaccharide composition of both EPS and CPS but no influence on advanced structure [25]. Although the dependence of synthesis and release of polysaccharide on light conditions in cyanobacteria has been widely reported, there are limited information about how this relevance is to be processed.

* Corresponding author.
E-mail address: jiashiru@tust.edu.cn (S. Jia).

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The understanding of the intracellular responses of *N. flagelliforme* to changes of light quality will give clues about the correlations between polysaccharide production and light conditions. Metabolomics, focusing on comprehensive analysis of metabolites in a biological system, has been established as a powerful tool to reveal the dynamic metabolic responses to environment disturbance [26,27]. To reveal the underlying relevance between polysaccharide production and light quality, the effects of light quality on the metabolic alterations were comprehensively studied using red, yellow, green, blue, purple light emitting diodes for illumination. The metabolic differences of *N. flagelliforme* grown under various light qualities were compared by metabolomic approach to elucidate the mechanism involved in the polysaccharide production.

2. Materials and methods

2.1. Strains and growth 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 a 1000 mL shake-flask containing 500 mL of medium at 25 °C under continuous illumination at a photon flux density of 60 μmol/(m^2 s) for monochromatic red (660 nm), yellow (590 nm), green (520 nm), blue (460 nm), and purple (400 nm) light. 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 fluorescent light were treated as control. The morphology of cells grown under different light qualities was observed by a light microscope (Olympus CX40, Olympus, Tokyo, Japan).

2.2. The measurement of elemental composition, photosynthetic activity, and production of EPS and CPS

The measurement of elemental composition was performed on a Flash 1112 Series elemental analyzer (Thermo Finnigan) according to the previously reported method with slight modification [28]. The photosynthetic and respiration rates of *N. flagelliforme* were measured by Clark-type electrode (Oxy-lab, Hansatech Instruments) as previously described [29]. The determination of EPS and CPS production was performed as previously reported [25].

2.3. Sample preparation and GC–MS analysis

Harvested samples were immediately quenched in liquid nitrogen and subsequently stored at −80 °C until further processing. Intracellular metabolites of *N. flagelliforme* were extracted as described by Lu et al. [30] and Han and Yuan [31] with slight modifications. Cells were ground into fine powder with a pestle and mortar. Then 100 mg cell powder was suspended in 1 mL of metabolite extract solution (methanol–water, 1:1 v/v, −40 °C) and mixed thoroughly, then the solution was frozen in liquid nitrogen and thawed for five times. After brief spinning separation, 200 μL of supernatant was freeze dried and 28 μg of succinic d4 acid (Sigma-Aldrich, Saint Louis, USA) was added as the internal standard. Derivatization of metabolites was accomplished via methoximation and trimethylsilylation. A 50 μL aliquot of methoxamine hydrochloride (20 mg L^{-1} in pyridine) (Sigma-Aldrich, Saint Louis, USA) was added to the dried sample and incubated at 40 °C for 80 min. After that, 80 μL of N-methyl-N-(trimethylsilyl) trifluoroacetamide (MSTFA) (Sigma-Aldrich, Saint Louis, USA) was added. The sample was incubated at 40 °C for an additional 80 min. The supernatant was transferred to gas chromatograph vials prior to GC–MS analysis.

Qualification and quantification of metabolites were performed on an Agilent Technologies 7890A gas chromatograph coupled to a series 5975C quadrupole mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) operated in electron ionization (EI) mode, which was equipped with a HP-5 capillary column (60 m × 320 μm i.d., 0.25 μm film thickness; Agilent J&W Scientific, Folsom, CA, USA). The temperature of the injector and the GC interface was maintained at 280 and 270 °C, respectively, while that of the ion source was set to 250 °C. Helium was used as the carrier gas under a constant flow rate of 1 mL min^{-1}. Ions were generated by a 70 eV electron beam and the mass range scanned was 50–800 m/z at a rate of 2 scan s^{-1}. The conditions for metabolite analysis were as follows: 1 μL sample was introduced with splitless injection. The oven temperature was initially maintained at 70 °C for 2 min and raised to 290 °C at a temperature rate of 5 °C min^{-1} and then held for 6 min.

Raw GC–MS data files were acquired by MSD Productivity ChemStation software (version E.0201.1177, Agilent Technologies, Palo Alto, CA, USA). Metabolites were identified by comparison of mass spectra of each compound to the National Institute of Standards and Technology mass spectral library (NIST, 2008, Gaithersburg, MD) using a cut-off value of 70%. A table providing relevant metadata of chromatography and mass spectrometry was shown in the Supplementary material (Table S1). The specific ion of each metabolite most suitable to distinguish the peak from local coeluting neighbor peaks was used for quantification. All metabolite data were normalized with the internal standard on the same chromatograph before further data analysis.

2.4. Statistics analysis

Experimental data in this study was obtained from at least three replicates for each treatment, and values were shown as mean ± standard deviation. Missing values were replaced by the lowest value of the respective metabolite in the data set ±40% random variation to keep the variance of the data set. Principal component analysis (PCA) and partial least-squares analysis (PLS) were performed with SIMCA-P Demo (Umetrics AB, Sweden) with pareto scaling prior to analysis. With Expander 4.1 software (EXpression Analyzer and DisplayER), the relative abundances of metabolites were standardized by mean 0 and variance 1 to generate a heat map.

3. Results and discussion

3.1. Effect of light quality on N. flagelliforme culture

We have previously reported the effects of light quality on *N. flagelliforme* polysaccharide production [25], and the work revealed that blue and red lights promoted EPS and CPS production compared with white fluorescent light and other monochromatic lights, indicating that light quality had important influences on both capsular and extracellular polysaccharide production. In this study, the effects of light quality on *N. flagelliforme* culture were further examined by comparing the elemental composition and photosynthetic activity of *N. flagelliforme* cells grown under various light conditions. As presented in Fig. 1A, among cells grown under various light qualities, there were no significant changes in C ratio, while the green and blue light-grown cells possessed higher ratio of N than cells grown under other light qualities, which subsequently caused the changes in C-to-N ratio in cells, indicating the differences in cellular components induced by light conditions.

The effects of different light quality on photosynthetic activity were presented in Fig. 1B. The red light-grown cells grew at a maximum photosynthetic rate of 159.65 μmol O_2 mg Chla^{-1} h^{-1} and highest respiration rate of 64.72 μmol O_2 mg Chla^{-1} h^{-1}, which were 1.30 and 2.02-fold to that of white light-grown cells. The observation was different from the performance of some higher plants and algae, the respiration of which could be activated by blue light [32]. The different performance in photosynthesis and respiration showed the difference in cellular state resulting from light quality, which was consistent with the result of the observed difference in cell growth [25] and cellular components.