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The regulation of photosynthetic pigments in terrestrial *Nostoc flagelliforme* in response to different light colors

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ABSTRACT

Cyanobacteria have evolved a number of photosynthetic strategies by adjusting the properties of the light-harvesting pigments to cope with changes in the light environment. *Nostoc flagelliforme* is a terrestrial filamentous cyanobacterium that has distinct phycobilisome composition consisting of phycocyanin (PC) and allophycocyanin (APC). The photoregulation process of *N. flagelliforme* was explored by investigating the effects of different light colors on photosynthetic pigments, which were determined after photoautotrophic growth with red (RL), yellow (YL), green (GL), blue (BL), purple (PL) and white (WL) light of same photosynthetically active photon flux density. Compared with WL, YL, GL, BL and PL changed the amounts of pigments but did not alter the cellular ratio of PC/APC; while RL altered both the amounts and proportions of PC and APC. The observations by confocal laser scanning microscope and transmission electron microscope showed that PC, APC and chlorophyll *a* were distributed throughout the cells under GL, BL, PL, and WL illumination but located in the outermost layer in RL and YL-grown cells. Further study revealed that changing RL intensity would not influence the ratio of PC/APC but the distribution of pigments shifted from central cytoplasmic region to the periphery of the cells with the increase of intensity. These results implied that *N. flagelliforme* responded to RL by regulating the composition and location of phycobilisomes and these findings would be of importance to develop the high-efficient process of *N. flagelliforme* culture.

1. Introduction

Environmental conditions exert a strong influence upon the cell growth, morphology and pigment composition of many photoautotrophic organisms [1–3]. As photoautotrophic organism, photosynthesis and growth rates are directly affected by light environment, because it is used to drive photosynthesis and regulate cell growth and development. Moreover, light can vary in terms of light quality, light quantity and photoperiod [4–6], thus sensing and adequately responding to light is a key attribute of eco-physiological versatility of photoautotrophic organisms.

Cyanobacteria, as photoautotrophic microorganisms, are one of the oldest groups of bacteria, which can date back to the Pre-Cambrian [7]. Until now, it has evolved a large variety of light-harvesting and protective pigments, and evolved many photosynthetic strategies to help them cope with changes in their light environment [8–10]. The regulation process of photosynthetic pigments to changes in light color not only affects the composition of phycobilisomes, but also has been reported to have significant influence on many cellular processes such as growth, photosynthesis, lipid production [1,11], nutrient utilization [12], and extracellular polysaccharides production [4]. The regulation process requires cyanobacteria to precisely measure ratios of specific light colors in its environment [13,14]. Besides the chlorophyll *a* (Chl *a*), cyanobacteria also contain several accessory pigments, including carotenoid and phycobilin, which can help them adapt to various light conditions. The phycobilins are unique among the photosynthetic pigments in that they are bonded to certain proteins, known as phycobiliproteins, which are commonly divided into three main groups: phycocyanin (PC), allophycocyanin (APC) and phycoerythrin (PE). Based on the shift in PC versus PE in the subunit structure of phycobilisome in response to light colors, four types of Chromatic Acclimation (CA) processes were identified till now [15].

Nostoc flagelliforme is a terrestrial filamentous cyanobacterium with great economic value that is mainly distributed in some arid and semi-arid areas of China, where they are often subjected to extreme environmental changes [16]. As a consequence, the characteristics of *N. flagelliforme* are quite different from the cyanobacterium in the aquatic environment, which shows strong ecological adaptability to

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extreme conditions such as alkali, desiccation and strong UV radiation [16–18]. In addition, the phycobilisome of *N. flagelliforme* contains only PC and APC [19], indicating the photoregulation process may be quite different from the reported CA types in cyanobacteria. Although there are more and more studies on CA in aquatic cyanobacteria [20–22], but for terrestrial *N. flagelliforme*, few reports about regulation process of photosynthetic pigments are available till now. Besides that, in our previous work investigating the effects of light color on *N. flagelliforme* culture, it was found that light color not only had strong influence on biomass accumulation, but also had obvious impact on cellular metabolism and polysaccharides biosynthesis [4,23]. Thus illustrating the responsive mechanism of photosynthetic pigments to the changes of light color is of great importance to understand the correlations between the photoregulation process and biomass accumulation and/or cellular metabolism. Therefore, the aim of this study was to research the photoregulation process of *N. flagelliforme* under light with different colors. Five different colors including red, blue, green, yellow, and purple were used and compared to white light (WL) of same photosynthetically active photon flux density. The content, composition, fluorescence properties, and localizations of photosynthetic pigments were comprehensively examined to study the alterations of photosynthetic pigments dependent on the changes of light color. The ultrastructure of *N. flagelliforme* cells grown under different light colors was also investigated.

2. Material and methods

2.1. Strain and culture conditions

The *N. flagelliforme* cells (TCCC11757) cultured in BG-11 medium were obtained from the Tianjin Key Lab of Industrial Microbiology (Tianjin, China). Inoculums were prepared regularly (bi-weekly) in 500 mL shake-flask under WL at a photon flux density of 60 μmol·photons/(m^2 s). Before all the experiments, the cells were incubated in a dark room for 3 days in order to reduce storage compounds, and then cells were cultured in 500 mL shake-flask containing 200 mL BG-11 medium at a photon flux density of 60 μmol·photons/(m^2 s). The initial pH of the medium was adjusted to 8.0 and the shake-flask was inoculated with 10% (v/v) cell suspension i.e. the initial cell density of cell culture was 0.37 g/L dry cell weight. For the cultivation with different colors of light, WL (390–770 nm) was provided by fluorescent lamps. Red (660 nm, RL), yellow (590 nm, YL), green (520 nm, GL), blue (460 nm, BL), and purple (400 nm, PL) lights with half-band width of 5 nm were provided by different light-emitting diodes (LEDs, Model no. CDL-CF22W, Shenzhen federal heavy secco electronic Co. LTD, China). Each LED light was composed of 22 colored bulbs and the wattage and voltage of each colored bulb was 1 W and 220 V. In order to avoid any interference from external light source, all experiments were done in a dark room. A quantum sensor connected to Light Scout Dual solar quantum light meter (Spectrum Technologies, USA) was used to measure the light intensity. The cells were cultured in air conditioned environment at 25 ± 1 °C and cells grown under WL were regarded as control. According to the growth curve of *N. flagelliforme* under different light colors as shown in Fig.S1, samples were taken at the middle of exponential phase and beginning of stationary phase for subsequent analysis of photosynthetic pigment and cell observation with spectral confocal laser scanning microscope (CLSM) and transmission electron microscopy (TEM).

2.2. The measurement of photosynthetic pigment

The content of photosynthetic pigment was determined as previously reported [24,25].

2.3. Cell observation with CLSM

An adequate amount of cells were taken and placed on the slide, which was covered by cover slip. CLSM (Hitachi F-4500, Tokyo, Japan) was used to observe the fluorescence of photosynthetic pigments in cells. To determine the optimal emission wavelength, the cells were excited with three individual laser wavelengths: 488 nm, 543 nm, or 633 nm. Subsequently the emission spectrum was gathered at 10.7 nm increments as a lambda Z-series.

For 488 nm excitation, the ArKr laser was set at 10% power, while for 543 nm and 633 nm excitation, the HeNe laser was set at 30% power and 10% power, respectively. The NT 80/20 filter was used to collect the images and emission scans were collected in ~10.7 nm bandwidth increments in the range from 500 to 740 nm. In order to obtain a better signal yield, scans were performed with 'low speed' setting (220 Hz, full scan mode) and the confocal pinhole was opened (600 mm diameter) for most measurements. The LSM FCS Zeiss 510 Meta AIM imaging software was used to obtain the acquired CLSM images.

2.4. Cell observation with TEM

A total of 10 mL of sample was taken and centrifuged at 8000 × *g* for 15 min, and the cell pellet was collected for subsequent analysis. The methods for chemical fixation, freeze substitution, processing and embedding of the samples were used as previously reported [26]. After that, the samples were viewed using the TEM (JEM-1230, Jeol Ltd., Tokyo, Japan) operated at 80 kV and equipped with a LaB6 filament. The Gatan 1.35 K × 1.04 K × 12 bit ES500W CCD camera was used to record all the micrographs.

2.5. Statistical analyses

Three replications from different batches of culture were performed for each experiment, and values were expressed as mean ± standard deviation. The Independent-samples *t*-test was used to analyze the experimental data using the SPSS statistical software (version 22.0) and the significance level was set at $P < 0.05$.

3. Results and discussion

3.1. The effects of light color on photosynthetic pigments

In order to explore the photoregulation process of *N. flagelliforme*, photosynthetic pigments contents under different light conditions were measured at exponential phase and stationary phase of growth, respectively. The experiments were conducted under six different light colors: RL, BL, GL, YL, PL as well as WL at a photon flux density of 60 μmol/(m^2 s). As shown in Fig. 1A, at exponential phase, the Chl *a* content was significantly higher under GL, WL, YL and lower under RL, than under BL and PL, while for carotenoid, it was significantly higher under PL and lower under RL, than under WL, YL, GL and BL. At stationary phase of growth (Fig. 1B), the Chl *a* content was higher in the order of GL, WL, YL, PL, BL and RL-grown cells with values of 4.61, 4.04, 3.97, 2.91, 2.88 and 0.98 mg/g, respectively; in comparison with exponential phase, the Chl *a* content remained almost unchanged, except for RL treatment, which was significantly decreased, while, the carotenoid content was slightly changed at all light conditions. The results showed that RL greatly reduced the Chl *a* content, and GL promoted the formation of Chl *a*, which corresponded with the findings of many reports [27,28]. Under PL, the highest carotenoid content was obtained with value of 1.84 mg/g, which was increased by 38.34% compared with control group. That might be explained by the fact that PL was harmful to the growth of *N. flagelliforme* [23], and carotenoid, as accessory pigment, could protect cell from light damage [29] besides the function of capturing light energy for use in photosynthesis.