

Effect of Light with Different Wavelengths on *Nostoc flagelliforme* Cells in Liquid Culture

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The effects of lights with different wavelengths on the growth and the yield of extracellular polysaccharides of *Nostoc flagelliforme* cells were investigated in a liquid cultivation. *N. flagelliforme* cells were cultured for 16 days in 500 ml conical flasks containing BG11 culture medium under $27 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of light intensity and 25°C on a rotary shaker (140 rpm). The chlorophyll *a*, phycocyanin, allophycocyanin, and phycoerythrin contents in *N. flagelliforme* cells under the lights of different wavelengths were also measured. It was found that the cell biomass and the yield of polysaccharide changed with different wavelengths of light. The biomass and the yield of extracellular polysaccharides under the red or violet light were higher than those under other light colors. Chlorophyll *a*, phycocyanin, and allophycocyanin are the main pigments in *N. flagelliforme* cells. The results showed that *N. flagelliforme*, like other cyanobacteria, has the ability of adjusting the contents and relative ratio of its pigments with the light quality. As a conclusion, *N. flagelliforme* cells favor red and violet lights and perform the complementary chromatic adaptation ability to acclimate to the changes of the light quality in the environment.

Key words: *Nostoc flagelliforme*, wavelengths, chlorophyll *a*, phycocyanin, allophycocyanin, phycoerythrin

Nostoc flagelliforme is an edible terrestrial cyanobacterium with great economic value. It has been used as a food delicacy for more than two thousand years, and its medicinal value has been recognized since ancient times [10]. In recent years, it has been reported that the hot water extract

from *N. flagelliforme* showed antitumor activity, and an acid polysaccharide named as Nostflan, which was isolated from the *N. flagelliforme*, displays extreme anti-HSV-I activity [13]. In China, *N. flagelliforme* is widely distributed in arid or semiarid areas, such as the western and northwestern regions [10]. However, it grows very slowly in the wild field and elongates only by 6% per year [6]. The increasing market demand in recent decades has resulted in the endangered status of this species and the deterioration of the environment. In order to protect the natural source of *N. flagelliforme* and the environment, many researchers tried cultivation of this species and a lot of techniques for the cultivation of *N. flagelliforme* have been developed, including cultivating it in the field [12], or its tissue in the sand [28], or cultivating the free cells in liquid culture medium [17, 25]. It was discovered that the growth rate of *N. flagelliforme* cells in liquid culture is much faster than that in the wild state. In addition, a large amount of extracellular polysaccharide is secreted to the liquid culture, and thus, both *N. flagelliforme* cells and extracellular polysaccharide can be obtained from the liquid cultivation [25]. In our lab, the photoautotrophic, mixotrophic, and heterotrophic cultivation of *N. flagelliforme* cells have been tried and it was found that the maximal photosynthetic rate, dark respiration rate, and light compensation point in mixotrophic culture were higher than those in photoautotrophic cultures [30]. The effects of some other factors on *N. flagelliforme* growth and properties have also been reported, such as the effect of solid bed-materials on its growth, morphology, and biological crust properties [5], the daily production and photosynthetic characteristics under ambient and elevated CO_2 conditions [21], the photosynthetic response to salt [29], the oxidative stress subjected by desiccation and effects of exogenous oxidants on its photosynthetic recovery [20], and *etc.*

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Light regulates the algal growth, polysaccharide production [27], ion transport [22], reproduction [16, 17], morphology [4], photosynthesis [23], metabolism, and gene expression [24], and lights with different wavelengths have different effects on algae. Many studies demonstrated that for most kinds of plants and green algae, the photosynthetic rate in the orange or red light is the highest, followed by that in blue or violet light, and that in the green light is the lowest [31]. There are also some articles about the effects of light on the growth and photosynthetic recovery of *N. flagelliforme* [8, 9]; however, little has been documented on the influences of light quality on *N. flagelliforme*. The aim of the present study was to investigate the effects of light with different wavelengths on the cell growth, and the accumulation of phycocyanin and polysaccharide in the liquid photoautotrophic cultivation of *N. flagelliforme* cells.

MATERIALS AND METHODS

Materials

N. flagelliforme (TCCC11757) utilized in liquid suspension cultures was isolated from a field colony that was collected on the eastern side of the Helan Mountain in Yinchuan, Ningxia, China, which was preserved in the Microbial Culture Collection Center of Tianjin University of Science and Technology. The culture medium utilized was BG-11 medium. One liter of BG-11 medium contains 75 mg $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 36 mg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.5 g NaNO_3 , 40 mg K_2HPO_4 , 6.0 mg citric acid, 6.0 mg ferric ammonium citrate, 1.0 mg EDTA, 20 mg Na_2CO_3 , 2.86 mg H_3BO_3 , 1.81 mg MnCl_2 , 0.22 mg ZnSO_4 , 0.04 mg Na_2MoO_4 , 0.08 mg CuSO_4 , and 0.05 mg $\text{Co}(\text{NO}_3)_2$. The water used was distilled water and all the reagents were of analytical purity and were bought from Tianjin Yuanli Chemical Co., Ltd. (Tianjin, China).

For the cultivation with different colors of light, white light (WL) was provided by fluorescent lamps (GB/T10682-2002; Shenyang Xinxing Industry Co., Ltd.). Blue (BL), green (GL), violet (VL), orange (OL), yellow (YL), and red (RL) lights were produced by using different broad-band filters (Weikang Colored Film Factory, Shanghai, China).

Culture Condition

The cultivations were conducted in 500 ml flasks (150 ml medium adjusted to $A_{750} = 1.0$) at $24 \pm 2^\circ\text{C}$, pH 9 under continuous illumination of $27 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ on a rotary shaker (140 rpm) for 16 days.

Light Treatments

The transmission spectra used for this study are presented in Fig. 1. Transmittance curves of the filters were measured with a UV-VIS spectrophotometer (TU-1810; Purkinje Genera, Beijing, China). Fine adjustments of the irradiance levels were performed by changing the number of light lamps and/or by varying the distance between the light sources and culture flasks. Irradiance was measured using a digital light meter (TES-1336A; TES Electrical Electronic Corp, Taiwan, China). The flasks were covered with the filters of cellophanes. The flasks were placed over the fluorescent lamps. The light intensities

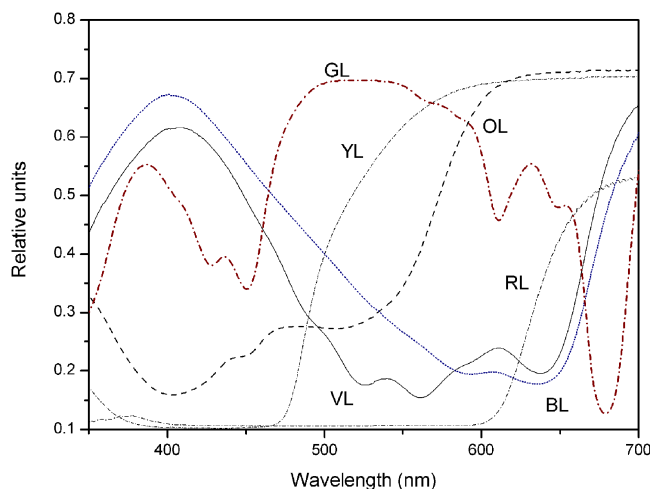


Fig. 1. Transmission spectra of the different-colored filters used for *N. flagelliforme* cells cultivation (BL: blue light; RL: red light; GL: green light; OL: orange light; YL: yellow light; VL: violet light).

were adjusted to $27 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$; the samples exposed to the white fluorescent lamps (390 nm–770 nm) were taken as the control.

Measurements of the Biomass, Polysaccharide, and Pigments

The biomass (cell concentration) of *N. flagelliforme* was determined by weighing the dry cells in 10 ml of culture medium after 15 min centrifuge under 4,000 rpm and drying at 105°C to constant weight (the centrifuge tube was pre-dried and weighed).

Chlorophyll *a* in the cell was extracted and determined according to the methanol extraction method [11]: 5 ml of sample was taken and centrifuged (LD5-2A; Medical Analytical Instrument Factory, Shanghai, China) at 4,000 rpm for 10 min. The supernatant was discarded. The algae was resuspended in 5 ml of methanol in a closed centrifuge tube and stored at 4°C overnight under dark conditions. Thereafter, the sample was centrifuged at 4,000 rpm and the absorbance of the supernatant at 665 nm was measured in a 1 cm cuvette using methanol as the blank. The chlorophyll *a* content was calculated as follows:

$$\text{Chl } a \text{ } (\mu\text{g/ml}) = 13.9 \times A_{665} \times V \text{ methanol}/V \text{ sample} \quad (1)$$

The extracellular polysaccharide content was determined *via* the modified phenol-sulfuric acid method [7].

In order to determine the contents of phycobiliprotein, 20 ml of *N. flagelliforme* culture medium was centrifuged at 5,000 rpm for 15 min. The cells were collected and then freeze-dried. The dried powder of *N. flagelliforme* cells was put into an agate mortar and then grinded for 30 min after some liquid nitrogen was added. Subsequently, a certain volume of distilled water was supplemented to dissolve the proteins. The mixture was transferred to a 10 ml centrifuge tube and centrifuged 5,000 rpm for 10 min and the absorbance of the supernatant was measured at 615, 652, and 562 nm, respectively, using a spectrophotometer (722; BOIF, Beijing, China). The phycobiliprotein contents were determined with the following equation [1]:

$$\text{CPC} = 0.0037 \times (A_{615} - 0.474A_{652}) \quad (2)$$

$$\text{CAPC} = 0.0039 \times (A_{652} - 0.208A_{615}) \quad (3)$$

$$\text{CPE} = 0.0021 \times (A_{562} - 2.41\text{CPC} - 0.849\text{CAPC}) \quad (4)$$

where CPC is the phycocyanin content, CAPC is the allophycocyanin content, and CPE is the phycoerythrin content. The total content of phycobiliproteins was the sum of the phycocyanin, allophycocyanin, and phycoerythrin contents.

RESULTS AND DISCUSSION

Effect of Light with Different Wavelengths on the Growth of *N. flagelliforme* Cells

Fig. 2 shows the biomass changes of *N. flagelliforme* cells with the cultivation time under the lights with different wavelengths. It can be seen that the biomass changed with the light source wavelengths, which demonstrated that *N. flagelliforme* had the wavelength selectivity to use the light sources. Under red and violet lights, the growth rates of *N. flagelliforme* were significantly higher than those under the lights of other wavelengths. After 16 days of cultivation, the dry cell weight came up to the highest value of 0.57 g/l under the red light, followed by violet and white lights, which were 0.5 g/l and 0.45 g/l, respectively. The biomass under the green light was the lowest at only 0.32 g/l. It can be considered that the red and violet lights are the most favorable light sources for the growth of *N. flagelliforme* cells, and the next in order is white light, but the green and blue lights are the most unfavorable.

Effect of Light with Different Wavelengths on the Yield of Polysaccharide

The yields of polysaccharide under different wavelengths of light are shown in Fig. 2. The wavelength of light

affected the yields of *N. flagelliforme* polysaccharide. The yields of polysaccharide under red and violet lights reached 41.38 mg/l and 44.76 mg/l, respectively, which were higher than those under the light of other colors and it is in accordance with the biomass changes.

However, there were differences between the effects of the light wavelength on the biomass, where the yield of polysaccharide under the violet light was the highest and that under the blue light was the lowest. That is, the violet light promoted the synthesis of *N. flagelliforme* polysaccharide the most.

Effect of Light with Different Wavelengths on Chlorophyll *a* and Phycobiliprotein Contents

Phycobiliproteins and chlorophyll are important pigments for *N. flagelliforme* cells. They play important roles in photosynthesis. Their contents affect the cell growth rate significantly. Chlorophyll *a* is a major photosynthetic pigment. It absorbs the light energy delivered to it by the photosynthetic reaction center. Phycobiliproteins including phycocyanin (PC), phycoerythrin (PE), and allophycocyanin (APC) are important auxiliary blue-green algae light-harvesting systems in photosynthesis.

Some studies [15–19] showed that light with different wavelengths can regulate the chlorophyll content in plants and the content varies in plant species, tissues, and organs. It can be seen from our experiments shown in Fig. 3 that the chlorophyll *a* content in *N. flagelliforme* cells changed with different wavelengths of light. The chlorophyll *a* content reached the highest of 10.91 mg/l in the red light, which was 1.4 times higher than that in the white light conditions, whereas *N. flagelliforme* cells cultivated in other wavelengths of light had less chlorophyll *a* contents than the control (white light).

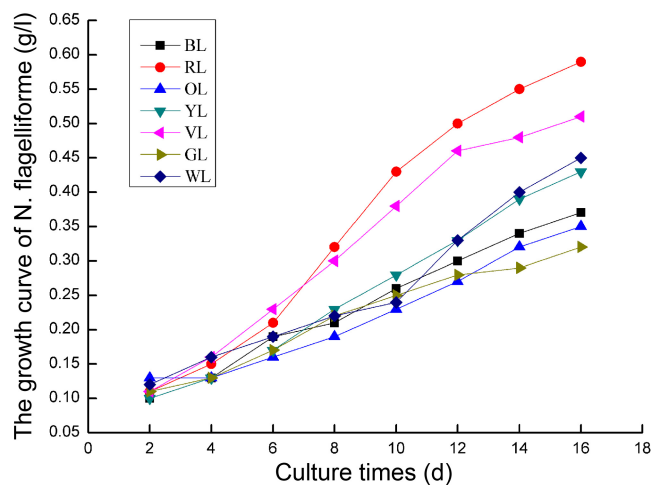


Fig. 2. The growth curve of *N. flagelliforme* under light with different wavelengths (BL: blue light; RL: red light; GL: green light; OL: orange light; YL: yellow light; VL: violet light; WL: white light).

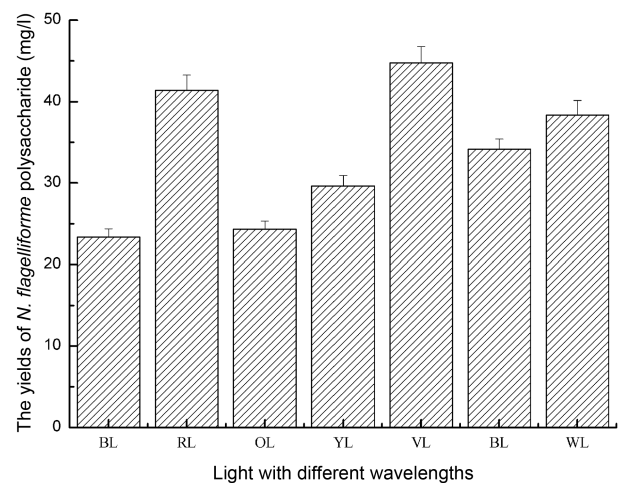


Fig. 3. The yields of *N. flagelliforme* polysaccharide under light with different wavelengths (All the acronym definitions here are shown in Fig. 2).

Table 1. Changes of phycocyanin, allophycocyanin, and phycoerythrin contents with light of different wavelengths.

Light source*	Phycocyanin	Allophycocyanin	Phycoerythrin	Total content of phycobiliproteins	Chlorophyll <i>a</i>
BL	5.89	3.12	0.67	9.68	6.56
RL	6.95	3.64	0.75	11.34	10.91
OL	4.34	2.67	0.51	7.52	5.57
YL	4.61	2.31	0.42	7.34	6.79
VL	6.56	3.71	0.61	10.88	7.86
GL	4.49	3.11	0.94	8.54	6.88
WL	6.45	3.45	0.81	10.71	8.01

*All the acronym definitions here are shown in Fig. 2.

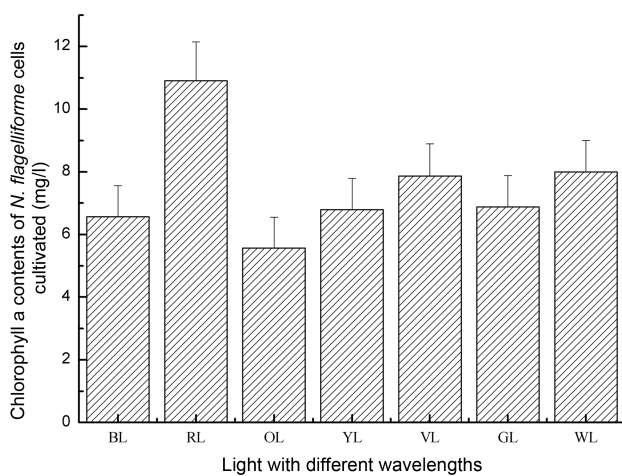


Fig. 4. Chlorophyll *a* contents of *N. flagelliforme* cells cultivated under light with different wavelengths (All the acronym definitions here are shown in Fig. 2).

By comparing the effect of lights at different wavelengths on cell growth and polysaccharide production of *N. flagelliforme* cells in liquid culture, it could be found that the cell chlorophyll *a*, phycobiliprotein content, extracellular polysaccharide production, and biomass were changed with the wavelengths of light during the cultivation.

Table 1 displays the effect of lights with different wavelengths on the contents of phycocyanin, allophycocyanin, and phycoerythrin in *N. flagelliforme* cells. It can be seen that the phycocyanin and the allophycocyanin contents reached the highest for the cultivations in the red and in the violet lights, respectively, but for the phycoerythrin content, it reached the highest in green light [2, 26]. This phenomenon showed the ability of complementary chromatic adaptation of *N. flagelliforme*, which is well known in other cyanobacteria and most phycobiliprotein-containing organisms [3]. This feature has not been documented for *N. flagelliforme*. *N. flagelliforme* cells adjust the phycobiliprotein composition in response to light quality, in order to make the most use of the light source to conduct photosynthesis and other life relating activities. Under red light, the

relative ratio of phycocyanin and chlorophyll *a* increased and their contents reached the highest, which intensified the absorption of the red light. It can be seen that chlorophyll *a* and phycocyanin with the main absorbances in the red light region are the main components in *N. flagelliforme* cells, which implies that *N. flagelliforme* tends to absorb the red light for photosynthesis and other life activities. However, for allophycocyanin, it is considered that its maximal absorbance is near 645 nm (red light region) [14], but its content reached the highest in the violet light. This phenomenon needs further investigation. Additionally, *N. flagelliforme* can enhance the absorption of green light by increasing its phycoerythrin relative ratio and content; however, because the content of phycoerythrin is comparatively less than the other pigments, the utilization of green light is less than red or violet lights. These indicate that the existence of the allophycocyanin and phycoerythrin gives *N. flagelliforme* cells an auxiliary ability to use the light of other colors, which improves the light efficiency for the photosynthesis and accumulation of biomass and polysaccharides. The *N. flagelliforme* cells not only tend to use red and violet lights for their cell growth and polysaccharide synthesis, but also show a complementary chromatic adaptation to acclimate to changes of ambient light conditions. Based on the yield of polysaccharide, biomass, chlorophyll *a*, and total pigment contents under different wavelengths of light, *N. flagelliforme* cells tend to make use of the red and violet lights. By adjusting the composition of the pigments, *N. flagelliforme* cells make the most use of the light sources.

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