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ORIGINAL PAPER

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Effects of intensity and quality of light on phycocyanin production by a marine cyanobacterium *Synechococcus* sp. NKBG 042902

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Abstract Among 150 strains, including marine cyanobacteria isolated from coastal areas of Japan and a freshwater cyanobacterium from the IAM collection, *Spirulina platensis* IAM M-135, the marine cyanobacterium *Synechococcus* sp. NKBG 042902 contained the highest amount of phycocyanin (102 mg/g dry cell weight). We have proposed that the cyanobacterium could be an alternative producer for phycocyanin. The effects of light intensity and light quality on the phycocyanin content in cells of *Synechococcus* sp. NKBG 042902 were investigated. When the cyanobacterium was cultured under illumination of 25 $\mu mol\,m^{-2}\,s^{-1}$ using a cool-white fluorescent lamp, the phycocyanin content was highest, and the phycocyanin and biomass productivities were 21 $mg\,l^{-1}\,day^{-1}$ and 100 $mg\,l^{-1}\,day^{-1}$ respectively. Red light was essential for phycocyanin production by this cyanobacterium. Phycocyanin and biomass production were carried out by the cyanobacterium cultures grown under only red light (peak wavelength at 660 nm) supplied from light-emitting diodes (LED). Maximum phycocyanin and biomass productivities were 24 $mg\,l^{-1}\,day^{-1}$ and 130 $mg\,l^{-1}\,day^{-1}$ when the light intensity of the LED was 55 $\mu mol\,m^{-2}\,s^{-1}$.

Introduction

Increasing attention has been paid to the application of marine biotechnological techniques (Matsunaga 1992). Marine microalgae can grow using CO_2, sunlight and other inorganic minerals present in sea water. Therefore, attention has been paid to the biotechnological potential of producing industrially useful chemicals using marine microalgae (Benemann et al. 1987; Pirt 1984), including work by our group (Burgess et al. 1993; Mastunaga et al. 1993, 1988, 1991; Miura et al. 1993; Takano et al. 1993; Wake et al. 1992b).

Cyanobacteria contain the phycobiliproteins phycocyanin, phycoerythrin and allophycocyanin as the light-harvesting apparatus. These phycobiliproteins have unique and high fluorescence properties, which make them attractive as diagnostic reagents and cosmetics (Glazer and Stryer 1984; Jassby 1988; Noue and Pauw 1988; Venkataraman 1989). Phycocyanin and phycoerythrin may be used as food dyes because of their intense color, their high solubility in water, and their stability to change in pH (Arad 1988), and the demand for phycocyanin for such applications is increasing. At present, most phycocyanin is commercially produced from the freshwater cyanobacterium *Spirulina platensis*. In this paper, we focus on phycocyanin production using a new isolate of a marine cyanobacterium. We have screened about 150 cyanobacterial strains isolated from Japanese coastal areas, specifically for their ability to produce phycocyanin.

Phycocyanin production is affected by various growth conditions (Babu et al. 1991; Ghosh 1966; Goedheer 1976; Jones and Myers 1965; Myers and Kratz 1955; Oquist 1974). The phycocyanin content of the cyanobacterium *Anacystis nidulans* increased after the shift from high intensity to low intensity, and white light to red light (Lonneborg et al. 1985). In the case of *S. platensis*, the phycocyanin content of the red-light-grown cells was greater than that of the cells grown on green light and same as that of the white-light-grown cells (Babu et al. 1991). Therefore, for the production of large quantities of phycocyanin, further investigation of the environmental effects of light quality is essential. In

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the case of *Synechococcus* sp. NKBG042902, red light appears to be essential for phycocyanin synthesis, as previously described (Babu et al. 1991; Lonneborg et al. 1985). In this report, we demonstrate phycocyanin production by this cyanobacterium using a novel light source based on light-emitting diodes (LED). Phycocyanin and biomass production were carried out by the cyanobacterium grown under red light.

Materials and methods

Strains

Marine cyanobacterial strains used in this study were isolated from coastal waters of Japan (collecting locations included: Onjuku, Izu, Miyazaki, Kagoshima, Saga, Nagasaki, Katsurahama, Okinawa, Ogasawara) and purified in our laboratory at Tokyo University of Agriculture and Technology. Collected samples, which were natural sea water and natural sea water containing macroalgae, were enriched and purified by agar plating. Artificial sea water (Matsunaga et al. 1988) and BG11 medium (Rippka et al. 1979) supplemented with NaCl (30 g/l) were used in the enrichment and purification process. Isolated cyanobacteria were assigned to genera according to the classification of Rippka et al. (1979).

Culture conditions of cyanobacteria

Cyanobacteria were cultured aerobically at 30 °C in a hexagonal flat flask containing BG11 medium (Rippka et al. 1979) supplemented with NaCl (30 g/l) under continuous illumination from fluorescent lamps (FL-20SS/18, Toshiba Co., Tokyo, Japan) at 25 $\mu mol\,m^{-2}\,s^{-1}$. *Spirulina platensis* IAM M-135 was obtained from the IAM collection (Institute of Molecular and Cellular Biosciences, University of Tokyo). *S. platensis* was cultured aerobically at 30 °C in a hexagonal flat flask containing SOT medium (Ogawa and Terui 1970) under the conditions described above. Cells grown on red (over 620 nm), green (peak wavelength at 530 nm) and blue light (peak wavelength at 440 nm) were exposed to the fluorescent lamp filtered through layers of appropriately colored cellophane papers wrapped around the culture flasks.

Construction of a culture system employing a novel light source based on LED

Cells were pre-cultured using a flat flask under continuous illumination from fluorescent lamps (FL-20SD-SDL, Toshiba Co., Tokyo, Japan) at 20 $\mu mol\,m^{-2}\,s^{-1}$ as the surface light intensity of the culture vessel. After harvesting by centrifugation, cells were resuspended in fresh medium. A schematic diagram of the culture system using red LED as a light source is shown in Fig.1. The red LED lamp was constructed with gallium aluminum arsenide chips as a double-heterostructure device and was a kind gift from Stanley Electric Co. Ltd. (Kanagawa, Japan). The spectral irradiance of light emitted from the red LED is shown in Fig.2. A red LED lamp unit was constructed from 80 lamps placed on an aluminum plate (1 cm × 23 cm) and the practical illumination area was 10.8 cm^2 (0.5 cm × 21.6 cm). Light intensity at the LED surface was controlled by operating at a constant voltage of 9 V and varying the forward current (0.05–1.2 A) from a d.c. power supply (Cross power 150, Atto Co. Ltd., Tokyo, Japan). The photon flux density ($\mu mol\,m^{-2}\,s^{-1}$) as light intensity was measured using a quantum sensor (LI-189, LI-COR, USA). The culture vessel was constructed from an acrylic flat box (10 cm × 28 cm × 3 cm) and a top plate, which were clamped together. The culture volume was 500 ml. Air was used for aeration at a flow rate of 500 ml/min. Cells were cultured under room temperature (25 °C).

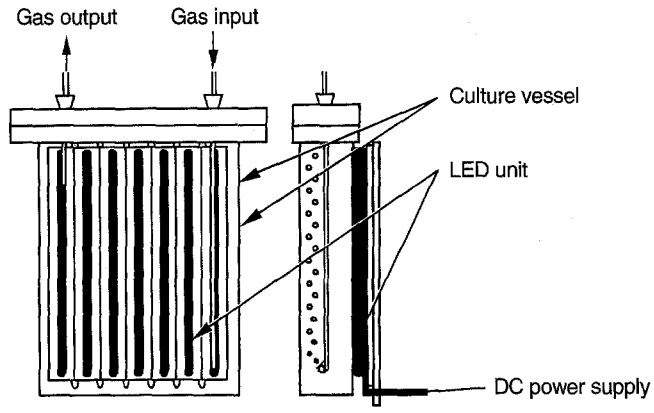

Fig. 1 Schematic diagram of a culture system employing red light-emitting diode (*LED*) lamps. Six units of LED lamps were positioned so that the emitted light was directed into the culture vessel. The total illumination area of the lamps was 64.8 cm^2

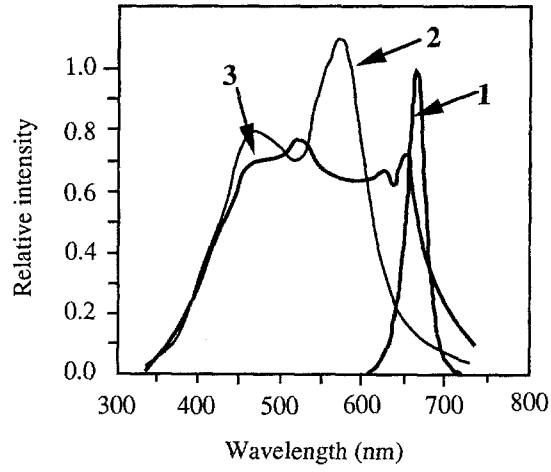

Fig. 2 Spectral irradiance of light emitted from the red LED lamps and from two fluorescent lamps used in this study. *1* A red LED lamp, *2* a fluorescent lamp (FL-20SS/18), *3* a fluorescent lamp (FL-20SD-SDL)

Analytical procedure

Biomass concentration in the culture suspension was determined as cell dry weight. Phycocyanin concentration was determined as previously described (Sode et al. 1991).

Results

Screening of marine cyanobacteria for phycocyanin production

We have screened 155 cyanobacterial strains isolated from Japanese coastal areas, specifically for their ability

Table 1 Phycocyanin content of various marine cyanobacteria. Cells were cultured at 30 °C under continuous light of 25 μmol $m^{-2} s^{-1}$ for 72 h

Strain	Phycocyanin content (mg/g dry cell weight)
Synechococcus sp.	
NKBG 040607	13.3
NKBG 041902	89.7
NKBG 031301	41.1
NKBG 042902	102.4
Chlorogloeopsis sp.	
NKBG 041302	35.8
Gloeothece. sp.	
NKBG 041101	3.2
NKBG 041607	1.4
Synechocystis sp.	
NKBG 000402	2.9
NKBG 040101	1.2
Myxosarcina sp.	
NKBG 041304	1.4
Dermocarpa sp.	
NKBG 041403	6.6
Other 144 strains	1.0–40.0
Spirulina platensis	
IAM M-135	90

to produce phycocyanin. Table 1 shows the phycocyanin content of various marine cyanobacteria isolated from coastal areas of Japan and a freshwater cyanobacterium *Spirulina platensis*. Most cyanobacteria contained 1–40 mg/g dry cell weight of phycocyanin. *Synechococcus* sp. NKBG 042902 contained the highest phycocyanin content (102 mg/g dry cell weight) among the isolated marine cyanobacteria and *S. platensis*. Therefore, for phycocyanin production, the *Synechococcus* strain can be recommended as an alternative to *S. platensis*. This strain could also grow faster than other strains. Growth and characteristics of this strain have been described previously (Matsunaga et al. 1990; Wake et al. 1991), but the effects of light intensity and quality on phycocyanin production by this strain were not known.

Effect of light intensity and quality on phycocyanin production

The effect of the light intensity on phycocyanin content was examined. The light intensity at the flask surface was set at 25 μmol $m^{-2} s^{-1}$ by using fluorescent lamps (FL-20SS/18), at which the phycocyanin content in the cell was maximum (Fig.3). The phycocyanin content in the cells cultured at 15 μmol $m^{-2} s^{-1}$ was almost the same as that at 25 μmol $m^{-2} s^{-1}$. However, the phycocyanin content in the cell decreased when the light intensity was increased from 25 μmol $m^{-2} s^{-1}$. The effect of light quality (red, green and blue) on the phycocyanin content in the cell was examined (Table 2). The phycocyanin content of cells grown on green light

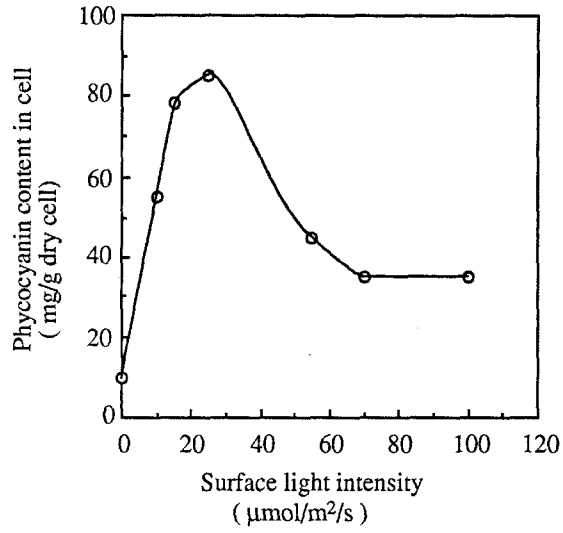

Fig. 3 Effect of light intensity on phycocyanin content in the cells of *Synechococcus* sp. NKBG 042902 irradiated by white fluorescent lamps. Cells were cultured in conical flasks at 30 °C for 72 h under continuous light

Table 2 Effect of light quality on phycocyanin content of *Synechococcus* sp. NKBG 042902. Cells were cultured at 30 °C under continuous light of 25 μmol $m^{-2} s^{-1}$ for 72 h

Light	Phycocyanin content (mg/g dry cell weight)
Red	63
Green	29
Blue	39

and blue light was markedly low. While that of the red-light-grown cells was high. Therefore, in the case of *Synechococcus* sp. NKBG042902, red light appears to be essential for phycocyanin synthesis. We supposed that the red light was an alternative and sufficient irradiation source for phycocyanin production by the marine cyanobacterium *Synechococcus* sp. NKBG042902.

Phycocyanin and biomass production by Synechococcus sp. grown under red light supplied from LED

The effect of light intensity at the surface of the culture vessel on phycocyanin and biomass productivity was examined (Fig. 4). Phycocyanin and biomass productivity were determined by the increase in amount of phycocyanin and cell dry weight in the medium after a 2-day culture period. Phycocyanin productivity gradually increased threefold when the surface light intensity at the culture vessel was increased from 5 μmol $m^{-2} s^{-1}$ to 55 μmol $m^{-2} s^{-1}$. The surface light intensity was set at 55 μmol $m^{-2} s^{-1}$, at which maximum phycocyanin productivity of 23 $mg l^{-1}$ day^{-1} was obtained. When the surface light intensity was

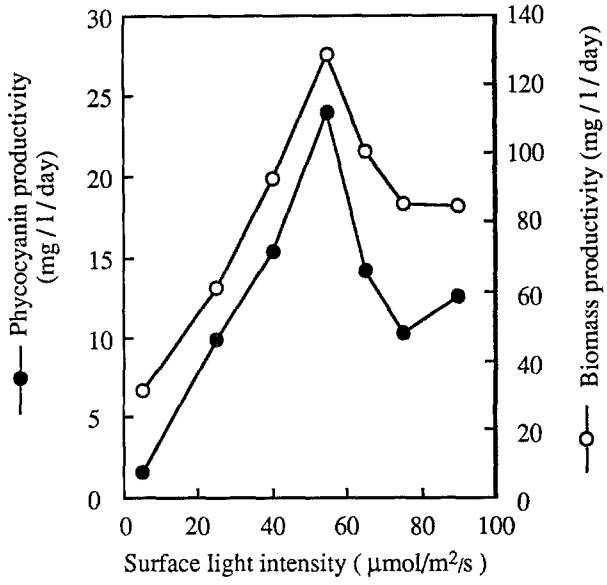

Fig. 4 Effect of light intensity on phycocyanin and biomass productivity by *Synechococcus* sp. NKBG 042902 irradiated by red LED lamps. Cells were cultured at 25 °C for 2 days under continuous light in the culture system described in Materials and methods

Table 3 Comparison of phycocyanin and biomass productivity by *Synechococcus* sp. NKBG 042902 culture grown under a red light-emitting diode (LED) lamp and a fluorescent lamp. Cells were cultured at optimum light intensities

Lamp	Phycocyanin productivity ($mg\,l^{-1}\,day^{-1}$)	Biomass productivity ($mg\,l^{-1}\,day^{-1}$)
Red LED[a]	24	130
Fluorescent[b]	21	100

[a] The optimum light intensity at the surface of the culture vessel was 55 $\mu mol\,m^{-2}\,s^{-1}$

[b] The optimum light intensity at the surface of the culture vessel was 25 $\mu mol\,m^{-2}\,s^{-1}$. Other culture conditions are described in Materials and methods

increased from 55 $\mu mol\,m^{-2}\,s^{-1}$ to 90 $\mu mol\,m^{-2}\,s^{-1}$, the phycocyanin productivity decreased. The profile of biomass productivity related to the surface light intensity was similar (Fig. 4).

Phycocyanin and biomass productivities by using a red LED and a cool fluorescent lamp were compared. When the white fluorescent lamp was used, an optimum light intensity of 25 $\mu mol\,m^{-2}\,s^{-1}$ for phycocyanin production was obtained (Fig. 3). On the other hand, the optimum light intensity of the red LED was 55 $\mu mol\,m^{-2}\,s^{-1}$. Therefore, the average phycocyanin and biomass productivities of the marine cyanobacterium *Synechococcus* sp. NKBG 042902 during the first 2-day culture period under both light conditions were compared (Table 3). Phycocyanin productivity of the cells cultured under the red LED lamp was 24 $mg\,l^{-1}\,day^{-1}$ and almost the same as that obtained by using the white fluorescent lamp. The biomass productivity by using the red LED was 130 $mg\,l^{-1}\,day^{-1}$, 1.3 times than that obtained when the white fluorescent lamp was used. Phycocyanin and biomass production was achieved by the cyanobacterium grown under red light.

Discussion

The cyanobacterium *Synechococcus* sp. NKBG042902 has been applied for CO_2 removal because of its greater ability to grow (Takano et al. 1992). It has also been reported that the cyanobacterium could produce novel plant growth regulators (Wake et al. 1992a, b, 1991) and, in this report, phycocyanin production by the cyanobacterium has been achieved. In the production of useful substances, it is an advantage to have several useful products and to grow faster. Therefore, the cyanobacterium has a great potential for producing industrially useful chemicals.

By comparing the phycocyanin contents in the cells of the marine cyanobacterium *Synechococcus* sp. NKBG042902 grown under three different qualities of light, the phycocyanin content in the red-light-grown cell was highest. It was previously shown that a shift from high intensity to low intensity, or from white light to red light could increase phycocyanin production by the cyanobacterium *Anacystis nidulans* (Lonneborg et al. 1985). In the case of *S. platensis*, the phycocyanin content of the red-light-grown cell was greater than that of the cells grown on green light and the same as that of the white-light-grown cells (Babu et al. 1991). However, changes in cell growth following such a shift were not described. The biomass productivity when the red LED was used was higher than that obtained with the white fluorescent lamp (Table 3). These results show that the red light radiation emitted from the red LED was sufficient for both growth and phycocyanin formation by the marine cyanobacterium *Synechococcus* sp. NKBG 042902. Production of other useful metabolites of *Synechococcus* sp. NKBG 042902 by using the red LED lamp is also under investigation.

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