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Comparison of pigments and photosynthate of *Nostoc* strains cultured photoautotrophically and chemoheterotrophically

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Abstract. *Nostoc* strains HN520 and HN701 grow equally well both chemoheterotrophically and photoautotrophically. Dark-grown cultures have significantly lower chlorophyll *a* and total phycobiliprotein contents than the light-grown cultures. Illumination was not rquired for the formation of either chlorophyll *a* or phycocyanin, but strain HN701 which contained phycoerythrin when grown in the light contained virtually no phycoerythrin when maintained in the dark. For both strains, the protein content decreased and the carbohydrate content increaed when they were gorwn in darkness. Growth in darkness did not affect the lipid content of strain HN520 markedly, but it decreased the lipid content of strain HN701 significantly. The fatty acid composition of both strains changed in darkness, but their amino acid compositions changed only slightly.

Key words: Amino acids; Carbohydrate; Chemoheterotropic growth; Cyanobacteria; Fatty acids; Lipids; *Nostoc;* Photoautotrophic growth; Pigments; Proteins.

Introduction

Some strains of photoautotrophic cyanobacteria are known to be able to grow in the dark (Fay, 1965; Cheung and Gibbs, 1966; Hoare *et al.*, 1971; Khoja and Whitton, 1971; Rippka, 1972; Sahu and Siba, 1982; Huang and Chow, 1988). When cyanobacteria are maintained in darkness, several of their physiological and biochemical properties probably change. Glucose incorporation and glucose-6-phosphate dehydrogenase activity of *Plectonema boryanum* increase during heterotrophic adaptation (Raboy *et al.*, 1976). The rate of lipid, RNA, protein and glycogen synthesis decrease when *Aphanocapsa* 6714 is transferred from photoautotrophic to dark heterotrophic condition (Pelroy *et al.* 1976). It is also known that the content of chlorophyll *a* and phycobiliproteins change as cyanobacteria are grown chemoheterotrophically (Hoare *et al.*, 1971; Sahu and Siba, 1982). Therefore, the chemical composition or even the metabolites of cyanobacteria could significantly change as they adapted to the dark heterotrophic condition.

The variation of property change of a cyanobacterium growing in darkness may depend on different isolates. In our previous study (Huang and Chow, 1988), several strains of *Nostoc* were found to grow very well heterotrophically in the dark. In this report, two of these isolates, *Nostoc* HN520 and HN701, were selected for a comparative study of their pigmentation, macromolecular contents and fatty acid and amino acid compositions when growing autotrophically in light and heterotrophically in the dark.

Materials and Methods

Organisms and Growing Conditions

Nostoc strains HN520 and HN701 were isolated

from local rice field and purified to be axenic in this laboratory. The morphology and some physiological properties of these isolates have been reported (Huang and Chow, 1988). BG-11_0, a nitrate-free BG-11 medium (Stainer *et al.,* 1971) was used for photoautotrophic growth. For the chemoheterotrophic growth, *Nostoc* HN520 was cultivated in BG-11_0+0.5% fructose, and strain HN701 in BG-11_0 + 0.5% glucose. Batches of autotrophic cultures (in 125 ml Erlenmeyer flasks) were incubated at 28°C with shaking under 35 μmol photons m^{-2} s^{-1} continuous white fluorescent light (Toshiba FL 20 D/18, Taiwan Fluorescent Lamp Co., Taiwan). The heterotrophic cultures were incubated at the same temperature, but in darkness.

Cells in the culture were collected by centrifugation. To cultures that were rich in mucilage, polyethylene imine (50% aqueous solution, Sigma) was added to have a final concentration of 5-10 mg/l to precipitate the cells before centrifugation. The collected cyanobacteria, after having been washed twice with liquid BG-11_0, were dried by lyophilization. Replicate cultures were mixed and grounded to a powder before being used for chemical analysis.

Extraction of Pigments

Chlorophyll *a* was extracted quantitatively with 100% methanol (Merck) at 60°C for 30 min and determined at 663 nm using specific absorption coefficient 78.74 l/g • cm (Codd *et al.,* 1980). Phycobiliproteins were extracted in 0.05 M potasium-phosphate buffer, pH 6.7. The concentrations of phycocyanin, phycoerythrin, and allophycocyanin in crude extracts were calculated according to the formula described by Siegelman and Kycia (1978). Absorption spectra of chlorophyll and phycobiliproteins were obtained on an Uvikon 810 spectrophotometer.

Quantification of Lipids, Carbohydrates and proteins

The content of macromolecular lipids, carbohydrates and proteins were determined according to a modification of the procedure described by Kochert (1978). Forty mg of lyophilized sample was twice extracted with 10 ml ice-cold 0.2 N $HC10_4$ in a 30 ml centrifuge tube to remove the low-molecular weight components. Lipids were then extracted with chloroform- methanol (1 : 1, v/v). The extract was transferred to a pre-weighed bottle and the solvent allowed to evaporate whereafter the residue was dried to constant mass in a vacuum descicator over P_2O_5 and NaOH pellets. The lipid yield was estimated by weighing the dried residue.

The sample, after lipid removal, was hydrolyzed with 3 ml of 1 N NaOH in a waterbath at 100°C for 10 min. The total protein content was determined by the biuret method (Gornall *et al.,* 1949) using bovine serum albumin as a standard. The total carbohydrate content was determined by the phenol-sulphuric acid method (Dubois *et al.,* 1956) using glucose as standard.

Determination of Fatty Acid Composition

About 0.5 g of lyophilized sample was used to extract the total lipids with ether. The saponification of the lipids and the acidification and methylation of the resulting fatty acids were performed according to the procedures described by Tseng *et al.* (1984). A Shimadzu GC-8A gas chromatograph equipped with a fused silica capillary column (HiCap-CBP-20, M25) was used to separate and determine the methyl esters of the fatty acids. The injection temperature was 220°C, and the column temperature 178°C. Nitrogen was used as carrier gas. Methyl esters of fatty acids obtained from Sigma were used as standards.

Determination of Amino Acid Composition

About 0.1 g dried cyanobacteria was hydrolyzed in a sealed ampoule with 1 ml of 6 N HCl at 110°C for 24 h. The HCl was eliminated under reduced pressure and the amino acid composition of the hydrolysate determined by means of a LKB 4150 Alpha Amino-Acid Analyser.

Results

Nostoc strain HN520 and HN701 respectively contained 1.09% and 0.99% chlorophyll *a* when they were grown in continuous light. The chlorophyll content decreased to 0.66% and 0.34% when they were grown heterotrophically in continuous darkness (Table 1). The total phycobiliprotein content changed from 6.56% and 5.52% respectively in the light grown HN 520 and HN701 strains to 3.71% and 4.42% respectively for the cells that were maintained in the dark (Table 1). When growing in light, the *Nostoc* HN520 cells consisted of 86% phycocyanin (PC) of the total phycobiliprotein content, a small amount of allophycocyanin (APC) and trace amounts of phycoerythrin (PE)

Table 1. *Chlorophyll **a**, phycobiliprotein, protein, carbohydrate and lipid contents of the light and dark grown cells of **Nostoc** HN520 and HN701*

Results presented are the mean values from 3 independent analyses.

Organism	Growth condition	Chlorophyll *a* (mg g^{-1} d wt)	Phycobiliproteins (mg g^{-1} d wt)				Proteins (mg g^{-1} d wt)	Carbohydrates (mg g^{-1} d wt)	Lipids (mg g^{-1} d wt)
			PC*	PE*	APC*	Total			
Nostoc HN520	Light	10.9	56.4	0.8	8.4	65.6	517	195	225
	Dark	6.6	30.5	2.2	4.4	37.1	367	307	235
Nostoc HN701	Light	9.9	23.2	29.2	2.8	55.2	438	231	255
	Dark	3.4	37.0	2.2	5.0	44.2	274	544	120

*PC, phycocyanin; PE, phycoerythrin; APC, allophycocyanin.

(Table 1). The ratio of PC : APC : PE did not change significantly when HN520 was grown in the dark, although the total content of these compounds decreased significantly (Fig. 1). As shown in Fig. 2, *Nostoc* HN701 contained more PE than PC when growing in light and consequently it had a brown colour. When it was grown in darkness, the PE almost completely disappeared, whereas the PC content increased by about 50% (Table 1 and Fig. 2). This large shift in the ratio of the phycobiliprotein components resulted in a change of the colour of strain HN701 from brown to deep blue-green when it adapted to growing in the dark.

The lipid, carbohydrate and protein contents of *Nostoc* HN520 and HN701 are also shown in Table 1. The protein content of both tested organisms growing in light was higher than when they were grown in the dark. The carbohydrate content, in contrast to the protein content, was lower in the light than in the dark. The lipid content of strain HN520 did not differ significantly for the light and dark treatments, but the lipid content of strain HN701 growing in the light was double that of the same organism growing in the dark.

The fatty acid composition of *Nostoc* HN520 and HN701 are shown in Table 2. *Nostoc* HN520 contained saturated, mono-unsaturated and some poly-unsatur-

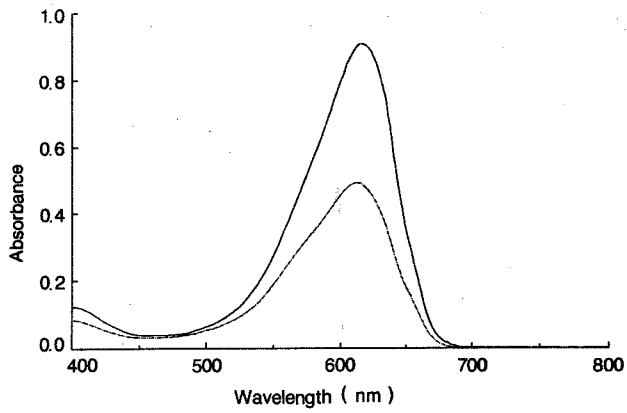

Fig. 1. Absorption spectra of the phycobiliproteins extracted separately from the same amount of light and dark grown cultures of *Nostoc* HN520. Both samples were extracted from 50 mg of the lyophilized cyanobacteria with 20 ml phosphate buffer. (————): Light grown culture; (— • — • — • —): Dark grown culture.

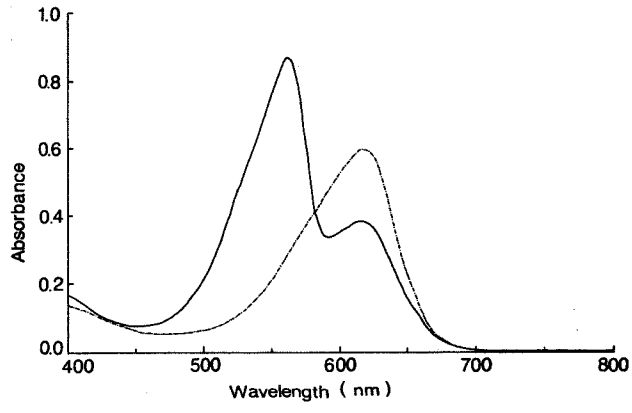

Fig. 2. Absoprtion spectra of the phycobiliproteins extracted spearately from the same amount of light and dark grown cultures of *Nostoc* HN701. Both samples were extracted from 50 mg of the lyophilized cyanobacteria with 20 ml phosphate buffer. (————): Light grown culture; (— • — • — • —): Dark grown culture.

Table 2. *The fatty acid composition of* ***Nostoc*** *HN520 and HN701 that were grown either in continuous light or darkness*

Numbers represent percentage of total fatty acid content. Each value is the mean of two independent determinations.

Fatty acid	*Nostoc* HN520		*Nostoc* HN701	
	Light	Dark	Light	Dark
$C_{14:0}$	trace*	1.6	7.3	5.8
$C_{15:0}$	trace	1.1	2.2	1.9
$C_{16:0}$	43.2	43.6	64.0	56.0
$C_{16:1}$	28.2	21.5	10.2	17.0
$C_{17:0}$	—	—	1.8	trace
$C_{18:0}$	trace	1.6	8.0	9.4
$C_{18:1}$	1.8	9.0	5.1	9.4
$C_{18:2}$	8.0	14.3	1.3	—
$C_{18:3}$	17.3	3.6	—	—
$C_{19:0}$	—	—	—	—
$C_{20:0}$	—	—	—	—
$C_{21:0}$	—	—	—	—

* trace : less than 1%.

Table 3. *The amino acid composition of* ***Nostoc*** *HN520 and HN701 that were grown either in continuous light or darkness*

Numbers represent percentage of total amino acid content (mole).

Amino acids	*Nostoc* HN520		*Nostoc* HN701	
	Light	Dark	Light	Dark
Try	—	—	—	—
Lys	4.1	3.9	4.2	4.7
His	1.2	1.3	1.3	1.4
Arg	10.1	11.5	5.7	5.4
Thr	5.4	5.1	5.8	5.4
Val	5.7	5.8	7.4	7.0
Met	0.9	1.5	2.0	1.4
Ile	4.7	5.4	6.6	5.8
Leu	7.9	7.5	10.7	10.0
Phe	3.5	4.6	3.4	3.6
Cys	—	—	—	—
Asp	17.0	17.6	10.9	11.4
Ser	4.6	4.0	5.1	4.2
Glu	9.9	7.8	10.2	11.2
Pro	4.5	4.1	3.9	4.4
Gly	8.0	7.3	8.1	8.1
Ala	10.2	9.8	12.2	13.1
Cysscy	—	—	—	—
Tyr	2.3	2.8	2.5	2.4

— : undetected.

ated fatty acids. The dark treatments affected the concentrations of the mono-unsaturated and poly-unsaturated fatty acids more than it did that of the saturated fatty acids. *Nostoc* HN701 contained almost exclusively the saturated and mono-unsaturated fatty acids, with the $C_{16:0}$ fatty acid as the major component. The treatments also had only minor effect on the fatty acid composition. The amino acid composition of the cultures is shown in Table 3. The dark treatments did not have a significant effect on the amino acid composition of the cells.

The morphology of the trichomes of strain HN520 and HN701 growing in the light and the dark are shown in Fig. 3. There was no difference except that the cells of the chemoheterotrophic culture was slightly larger than those of the photoautotrophic culture.

Discussion

The pigment, protein, carbohydrate, and lipid contents of both *Nostoc* HN520 and *Nostoc* HN701 were markedly affected when they were grown chemoheterotrophically. The morphology of the trichome, on the other hand, was not affected except that the cell size was slightly influenced. After more than half a year of chemoheterotrophic growth the culture still contained fairly high concentrations of chlorophyll *a* and phycocyanin. Therefore illumination is not required for the formation of chlorophyll *a* and phycocyanin of *Nostoc* HN520 and HN701. The phycoerythrin content is known to be affected by light quality and intensity (Bennette and Bogorad, 1973). Phycoerythrin is the dominant phycobiliprotein in light-grown *Nostoc* HN701 but disappeared almost completely when strain HN701 was grown in the dark. This result agrees with the data obtained by Sahu and Siba (1982) who observed that PE is not produced in dark-grown cultures of *Anabaena* sp. and *Calothrix marchica.* This indicates that the photoregulation of the biosynthesis of PC and PE differs.

Nostoc has a potential to form hormogonia (Rippka *et al.,* 1979). The ratio of hormogonia: heterocystous filaments usually increase when a culture ages. *Nostoc* HN701 had a stronger tendency to form hormogonia than *Nostoc* HN520 or the other *Nostoc* strains isolated in this laboratory. After a HN701 culture had been growing in the $BG\text{-}11_0$ medium for more than three weeks under continuous illumination, almost

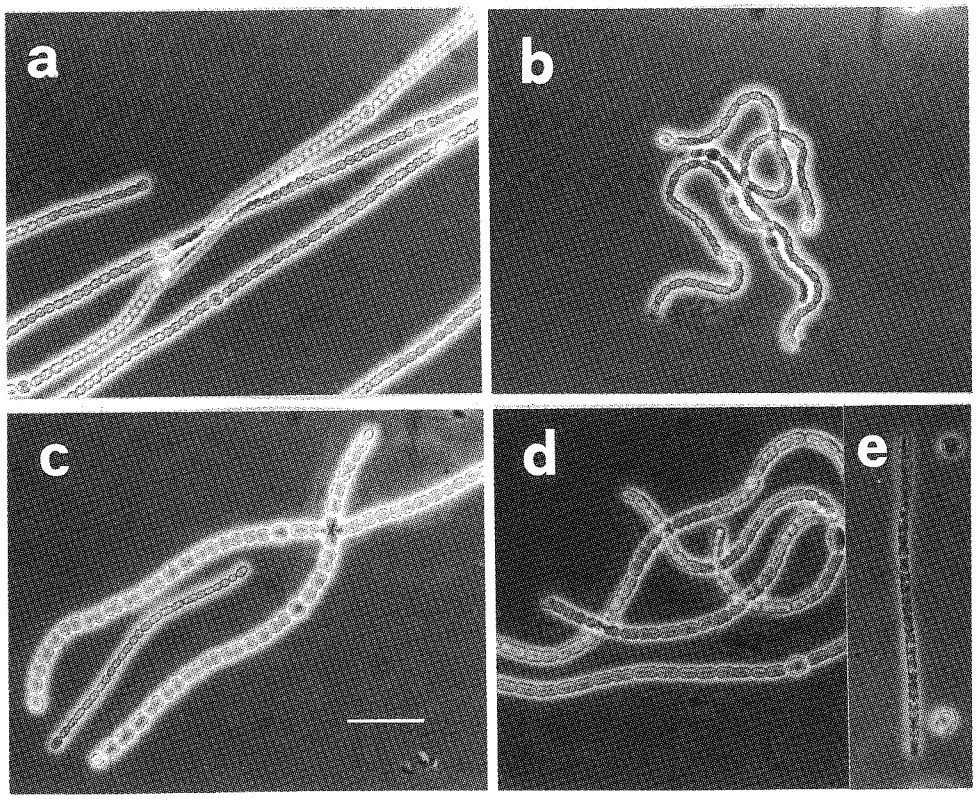

Fig. 3. Micrographs of *Nostoc* HN520 and HN701. (a): Strain HN520 grown in light; (b): Strain HN701 grown in light; (c): Strain HN520 grown in dark; (d): Strain HN701 grown in dark; (e): The hormogonia of strain HN701 transformed from the dark grown culture. Magnification the same in all cases. Bar = 20 μm.

all the trichomes will have transformed into hormogonia. Therefore, the culture of HN701 used for chemical analysis had to be collected before it was transformed into homogonia.

As shown in Table 2, *Nostoc* HN701 contained small amounts of the $C_{17:0}$ fatty acid—a fatty acid which does not commonly exist in cyanobacteria. Based on several independent experiments, it was also found that the $C_{17:0}$ fatty acid content varied much more significantly than did the content of the other fatty acids (unpublished data) Since it is difficult to obtain a hormogonia-free culture of HN701, and the size of the hormogonia population usually differed from culture to culture, the fatty acid composition of the hormogonia of strain HN701 was analyzed separately. As shown in Fig. 4, the fatty acids were mainly $C_{17:0}$ fatty acid. The results suggest the small amount of the $C_{17:0}$ fatty acid that was obtained from the light-grown HN701 strain may have been due to the presence of hormogonia in the culture.

The great diversity and consistency of the fatty-acid pattern of cyanobacteria make it to be a useful criteria for classification (Holton *et al.*, 1968; Kenyon and Stanier, 1970). In general, the unicellular strains of cyanobacteria have only saturated and monounsaturated fatty acids (Kenyon, 1972), whereas the filamentous type usually contain polyunsaturated fatty acids (Holton *et al.*, 1968; Kenyon and Stanier 1970; Kenyon *et al.*, 1972). Kenyon (1972) has shown that the fatty acid composition of several unicellular cyanobacteria are not affected by the growth medium, light source or temperature. De Loura *et al.* (1987) also find that under nitrogen deficient condition, the fatty-acid patterns of *Pseudanabaena* sp. and *Oscillatoria splendida* remain unchange. The data of this study indicated that the fatty acid composition of *Nostoc* HN520 and HN701 were changed when growing under dark heterotrophically. However, the changes were relatively minor when it was compared to the changes of pigments and macromolecular contents.

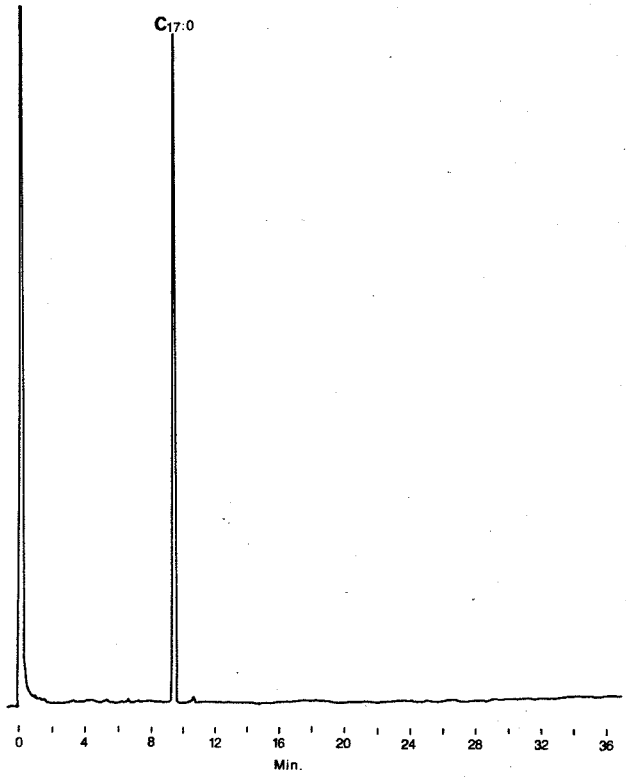

Fig. 4. Gas chromatogram of methyl esters of the saponifiable fatty acids extracted from the hormogonia of strain HN701 cultivated under light.

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比較念珠藻品系在光合自營與化合異營條件下之色素和光合產物

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念珠藻 HN520 和 HN701 兩品系在光合自營與化合異營條件下均生長良好。兩者之葉綠素 a 和藻藍蛋白在黑暗培養時均可產生，但對於含藻紅蛋白 之HN701 品系，在黑暗培養時其藻紅蛋白完全消失。在含量變化上，生長於黑暗時，兩者之葉綠素 a 和藻膽色蛋白的含量均比生長於光照時低，同時蛋白質含量也下降，但醣類則增加。黑暗處理並不影響 HN520 品系之脂質含量，但却使 HN701 品系之脂質含量下降。此外，黑暗處理會引起脂肪酸組成的改變，但胺基酸組成則無明顯變化。