J. Phytol. Res. 10 (1-2): 125-131, 1997

PHOTO-ASSIMILATION, GROWTH AND NITROGEN FIXATION OF A HETEROCYSTOUS NITROGEN - FIXING CYANOBACTERIUM *CAMPTYLONEMA INDICUM* ON ORGANIC SUBSTANCES AS CARBON SOURCE

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A heterocystous, filamentous cyanobacterium *Camptylonema indicum* was studied to observe the uptake of organic carbon sources like acetate, pyruvate and glucose on growth and nitrogen fixation under photoheterotrophic conditions. It was found that growth, nitrogen fixation, cell nitrogen as well as uptake of organic carbon source under photoheterotrophic conditions varied greatly.

Keywords: *Camptylonema indicum;* Nitrogen fixation; Organic carbon source, Photoassimilation.

Introduction

Most of the cyanobacteria are in general obligate photoautotrophs; but several strains can support growth on exogenously supplied organic carbon source in light, which provide carbon skeletons and ATP requirements[1,2]. Nitrogen fixation in cyanobacteria require an adequate supply of ATP and reducing power for production of organic nitrogen. This paper deals with possible effect of organic carbon source on pboto-assimilation (chemoheterotrophy) heterocyst differentiation, cell nitrogen absorption spectra and total chlorophyll content under various light intensities and wavelenghts provided in *Camptylonema indicum.*

Materials and Method

The cyanobacterium *Camptylonema indicum* was grown axenically in Allen & Arnon's[3] nitrogen-free medium unless otherwise mentioned. pH of basal medium was adjusted to 7.8, after autoclaving. Organic carbon source selected for study were acetate as sodium acetate; pyruvate as sodium pyruvate and D-glucose. The basal medium contained organic carbon source as 1 mg/ml which was determined after preliminary experiments. Different light intensities used were 600, 1200, 2400, 3600 and 4600 lux, using incandescent lamps in closed cabins at temperature of 37 ± 5° C, growth experiments were carried out in corning glass tubes (60 ml) and these were hand-shaken 3-4 times daily during the experimental growth period of 20 days. Growth was measured in terms of optical density at 660 nm of acetone - soluble pigments, dry weight and total chlorophyll-a content. Absorbance studies were performed using Zeiss - spekol spectrophotometer. Total cell protein was determind by Lowry *et al.*[4] method. Blue, red, green and white light conditions were created by wrapping the tubes with cellophane paper of respective colour and incubating them in culture chamber. Heterocyst frequency is expressed as number of heterocysts per 100 vegetative cells by counting 20 filaments. All the observations represent a mean of 4 values.

Results and Discussion

Growth of culture under nitrogen-fixing conditions with various organic carbon sources under photoheterotrophic conditions was measured after 20 days. In 600 lux of light there was appreciable growth in pyruvate

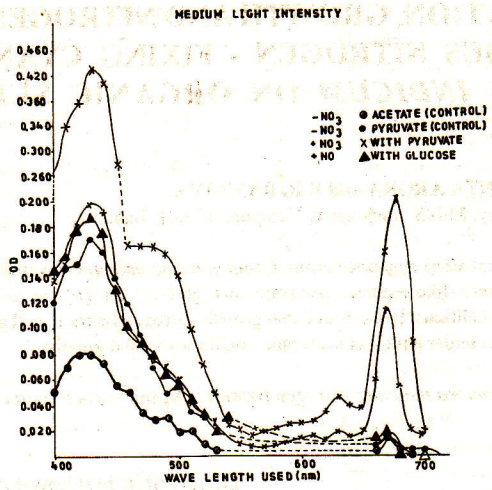

Fig. 1. Absorption spectra of chlorophyll pigments of *C. indicum* grown in medium light intensity (2400 lux). Symbols used:
Θ + Acetate $-NO_3$; • + Pyruvate $-NO_3$
X + Pyruvate $+NO_3$; ▲ + Glucose $+NO_3$

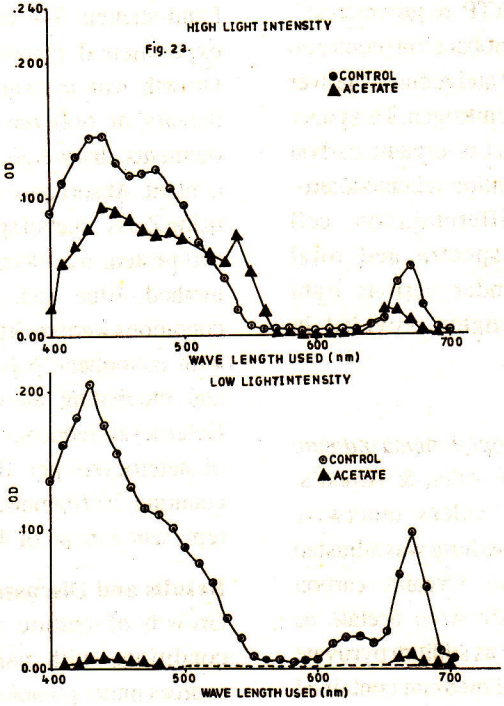

Fig. 2. Absorption spectra of chlorophyll pigments of *C. indicum* under high (2a) (3600 lux) and low (2b) light intensity (600-1200 lux).
Θ Control ▲ Acetate.

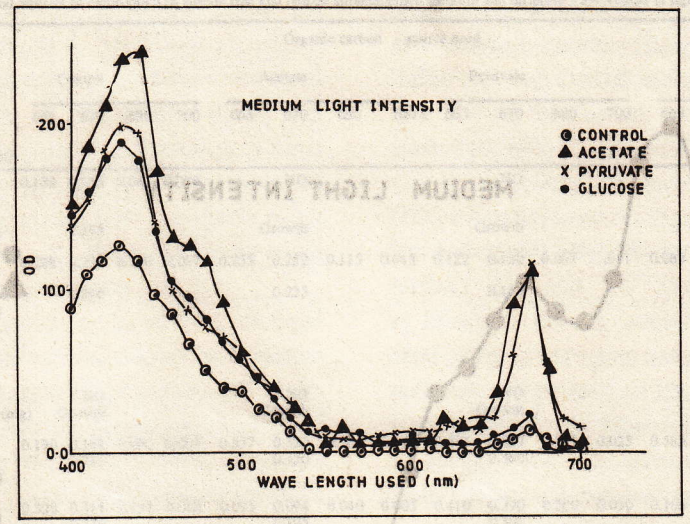

Fig. 3. Absorption spectra of chlorophyll pigments of *C. indicum* grown in medium light intensity (2400 lux). Θ Control; ▲ Acetate; X Pyruavte; • Glucose.

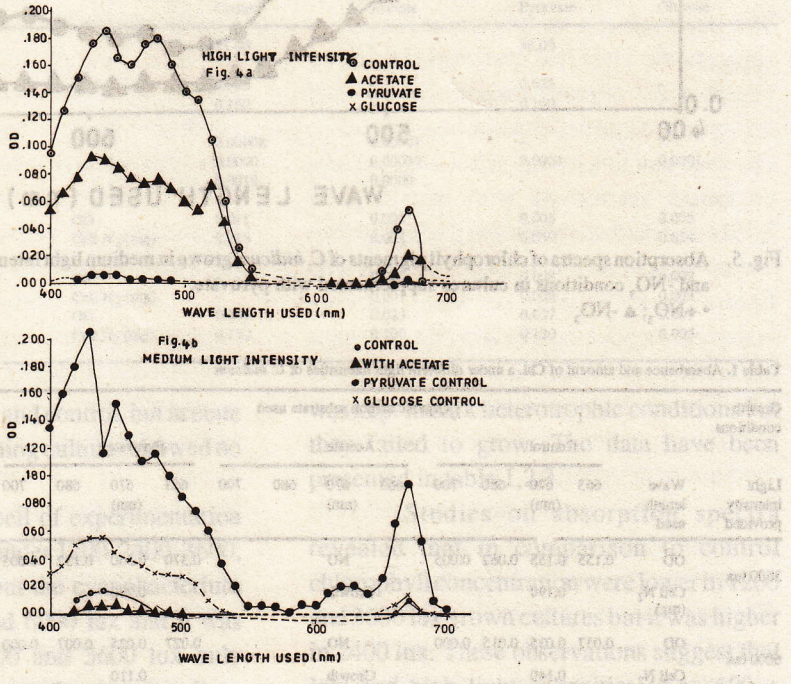

Fig. 4. Absorption spectra of chlorophyll pigments of *C. indicum* in high light intensity (3600 lux) (4a) and medium light intensity (2400 lux, 4b).
Θ Control; ▲ Acetate; • Pyruavte; X Glucose.

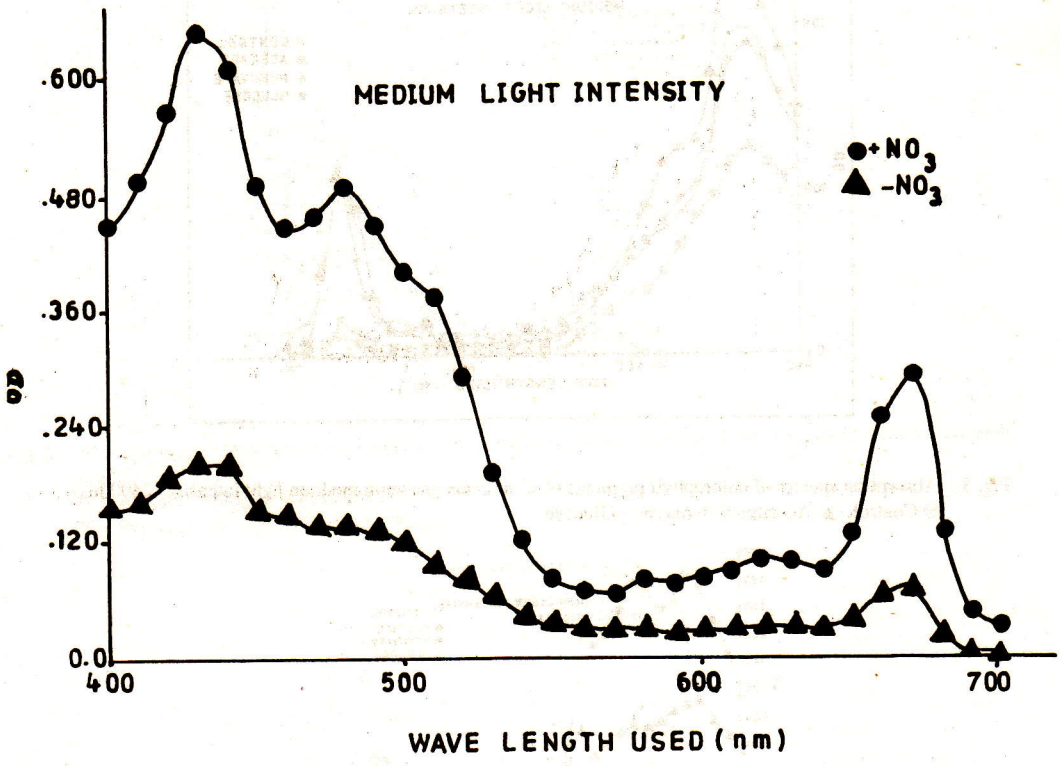

Fig. 5. Absorption spectra of chlorophyll pigments of *C. indicum* grown in medium light intensity (2400 lux) under $+NO_3$ and $-NO_3$ conditions in cultures supplemented with pyruvate.
• $+NO_3$; ▲ $-NO_3$

Table 1. Absorbance and amount of Chl. a under different light intensities of *C. indicum*

Growth conditions		Organic carbon substrate used															
		Control				Acetate				Pyruvate				Glucose			
Light intensity provided	Wave length used	663	670 (nm)	680	700	663	670 (nm)	680	700	663	670 (nm)	680	700	663	670 (nm)	680	700
3600 lux	OD	0.155	0.155	0.062	0.005		NO			0.370	0.360	0.132	0.005	0.195	0.187	0.078	0.002
	Cell N_2 (mg)		0.190				Growth				0.290					0.210	
6000 lux	OD	0.037	0.035	0.015	0.000		NO			0.027	0.025	0.007	0.000			NO	
	Cell N_2 (mg)		0.140				Growth				0.110					Growth	
8000 Lux	OD		NO				NO				NO					NO	
	Cell N_2 (mg)		Growth				Growth				Growth					Growth	

Table 2. Absorbance and amount of chlorophyll in Nitrate free and Nitrate supplemented medium and different wavelengths of light in *C. indicum*

		Organic carbon source used															
		Control				Acetate				Pyruvate				Glucose			
	Wave length used (nm)	663	670	680	700	663	670	680	700	663	670	680	700	663	670	680	700
AA-NO_3 medium	OD	0.158	0.155	0.065	0.006		NO				NO				NO		
	Cell N_2 (mg)		0.195				Growth				Growth				Growth		
AA+NO_3 medium	OD	0.288	0.258	0.150	0.007	0.255	0.252	0.115	0.015	0.122	0.120	0.057	.011	0.088	0.082	0.37	0.010
	Cell N_2 (mg)		0.240				0.235				0.145				0.190		
Light Provided																	
Blue	OD		NO				NO				NO				NO		
	Cell N_2 (mg)		Growth				Growth				Growth				Growth		
White	OD	0.190	0.188	0.080	0.005	0.557	0.520	0.265	0.027	0.587	0.535	0.295	0.025	0.585	0.533	0.290	0.052
	Cell N_2 (mg)		0.215				0.350				0.380				0.400		
Red	OD	0.320	0.315	0.133	0.020	0.095	0.094	0.040	0.005	0.410	0.390	0.200	0.030	0.105	0.103	0.050	0.012
	Cell N_2 (mg)		0.270				0.195				0.300				0.225		

Table 3. Amount of dry weight, Cell N_2, total Chl. a and optical density of *C. indicum* under different light intensities and wave lengths used in *C. indicum*

			Organic carbon source used			
			Control	Acetate	Pyruvate	Glucose
Growth Parameters taken	Dry wt. (mg)		53.00	-	38.00	-
	OD		0.040	-	0.025	-
	Cell N_2 (mg)		0.160	-	0.100	-
Light intensity used	3600 lux		0.00008	0.00001	-	-
	2400 lux		0.0000	0.00032	0.0004	0.0001
	1200 lux		0.0019	0.0000	-	-
Wave length of light used	Red	OD	0.011	0.000	0.005	0.005
		Cell N_2 (mg)	0.080	0.004	0.050	0.054
	Green	OD	-	0.000	0.008	0.002
		Cell N_2 (mg)	-	0.001	0.008	0.003
	White	OD	0.039	0.023	0.027	0.009
		Cell N_2 (mg)	0.130	0.100	0.120	0.005

supplemented culture and control, but acetate and D-glucose containing cultures showed no growth.

In second spell of experimentation the cultures were put under 1200, 2400, 3600, 6000 and 8000 lux, but the cyanobacterium failed to grow beyond 6000 lux and it was observed that in 1200 and 3600 lux only control and acetate supplemented culture could grow and under 2400 lux all the cultures showed measurable growth. One set of above was kept in dark heterotrophic conditions but they failed to grow. The data have been presented in table 1,2,3.

Studies on absorption spectra revealed that in comparison to control chlorophyll concentration were lower in 1200 and 3600 lux grown cultures but it was higher in 2400 lux. These observations suggest that low and high light intensities (e.g., 600 + 1200 lux referred as low and 3600 lux as high and 2400 lux as medium in this paper) were

not conducive to growth and nitrogen fixation and uptake of organic carbon substances was maximum in medium light (Fig.1-5). Further acetate supported maximum growth and provided carbon skeletons for cell constituents followed by pyruvate and glucose.

When cultures were put under 2400 lux and various wavelengths (red, green and white) of light on organic carbon supplemented media, growth occurred in white light only, but under red or green light it was poor growth; supporting the view that obligate photoautotrophy still play a dominant role in cyanobacteria, even when uptake of exogenously supplied organic carbon takes place on a limited scale (Table 3).

The heterocyst frequency was increased by D-glucose, pyruvate and acetate from 5.6 to 7%, 8% and 8.5% respectively. The observed rise in heterocyst frequency supports the view that carbon skeletons are supplied by organic carbon source; and incorporated into cell constituents. It was also observed that low carbon containing cultures contained heterocysts with normal polar bodies and thick envelops, but the high carbon containing cultures had long filaments with heterocysts poorly formed, lacking conspicuous polar bodies and thin envelops; again emphasizing the view as given in above para. There is increase in chlorophyll-a content and protein content in these cultures under medium light intensity.

Many cyanobacteria are unable to grow in dark on organic carbon sources, but nevertheless they may utilize them in light[5]. The dark anaerobic metabolism of *Nostoc* sp. strain Cc show on transfer actively metabolise glucose to yield acetate thereby show fermentation[6].

Photo-assimilation of acetate which has been shown in *Nostoc muscorum*[7], *Anabaena variabilis*[8]. It might be expected from other kind of cyanobacteria and algae that photo-assimilation of organic carbon source at limiting light intensity would increase relative growth rates higher than those which can be achieved with CO_2, but, alone would not result.

All the organic compounds that support growth of cyanobacteria can be metabolised via oxidative pentose-phosphate cycle. The exception to this generalisation are reports on growth of *Chlorogloeopsis* 6912[9]. Oxidative pentose-phosphate pathway is the route for aerobic metabolism of exogenously supplied glucose[10]. While transport mechanism in *Synechocystis* 6714 and *Nostoc* strain MAC were of similar activity in organisms grown in light or dark, glucose incorporation in *Plectonema* 73110 was induced sevenfold on transfer from photoautotrophic to chemoautotrophic conditions.[10] From analogue uptake studies and other features it has been concluded that these heterotrophic cyanobacteria possess mechanisms for active transport of organic growth substrates into the cell.

The accumulation of exogenous organic substrates with prokaryotic cells at concentrations greater than in medium is necessarily dependent on a source of energy under photoheterotrophic conditions; this energy is provided by cyclic photophosphorylation whereas in dark substrate utilization is coupled to the catabolism of carbohydrate obtained from the medium[11].

Photoautotrophic growth is predominant metabolic ability of cyanobacteria and under conditions that sustain high rate of CO_2 fixation, the

assimilation of exogenous organic compounds usually make only a small contribution to synthesis of cell material[12].

Natural populations of cyanobacteria have been found where there is a little if any light. In sediments or reservoirs and lakes and aphotic zone of sea, metabolism of cyanobacteria is likely to be heterotrophic and any growth would be dependent on exogenous compounds.[1,13].

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