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GROWTH OF CYANOBACTERIA IN PRESENCE OF ORGANIC CARBON SOURCE

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Two cyanobacteria *Anabaena doliolum* and *Nostoc muscorum* were grown photoheterotrophically in sucrose supplemented medium under continuous irradiation and nitrogen-fixing conditions. Growth responses of both cyanobacteria were studied using growth parameters, viz., absorbance, total chlorophyll-a, protein content, pH and conductivity measurements. Heterocyst frequency, spore germination and sporulation were also estimated. Ammonical nitrogen uptake was determined in ammonical nitrogen supplemented medium.

Keywords : *Anabaena doliolum;* Cyanobacteria; Growth; Nitrogen; Sucrose.

Introduction

Most cyanobacteria, in general do not grow heterotrophically, but in recent years few species of cyanobacteria have been reported to be capable of growing slowly in dark/light when supplied with suitable organic carbon and nitrogen sources[1-3], which provide both carbon skeletons and energy. All the information available on this aspect of nutrition, development and differentiation indicate that a close relationship between cellular differentiation and metabolism of nitrogen and carbon compounds occur in cyanobacteria[2,4,5]. The exogenous substrates used are quite specific and the extent of their specificity to accelerate growth of cyanobacteria varies with different genera, species and strains[3,4].

Present investigation deal with the effect of sucrose on growth and development of two cyanobacteria *Anabaena doliolum* and *Nostoc muscorum* under phototrophic conditions, using photosynthetic inhibitors and stressful concentration of sucrose into the growth medium. This would help to understand the growth behaviour and osmotic adjustment by cyanobacteria, under photoheterotrophic environment.

Materials and Method

Two cyanobacterial taxa used in present study are *Anabaena doliolum* and *Nostoc muscorum* isolated from rice-fields of Dungarpur and Banswara region of Udaipur division. Cultures were made bacteria - free using standard procedures and maintained on Allen and Arnon's[6] nitrate - free medium. Experiments were conducted in 60 ml corning glass culture tubes, using 15 ml basal medium in each tube. Growth responses of above test-organisms were observed by growing cyanobacteria into different concentrations of sucrose supplemented medium (pH 8.0), for 18 days under continuous fluorescent light (2000 lux) at 30±2°C.

Growth parameters studied were OD, total chl- a, protein content[7], pH and conductivity values. Absorption - spectra of cultures were recorded which contained upto 0.6 M of sucrose in basal medium. Heterocyst frequency was determined as number of heterocysts per 100 vegetative cells using 10 fields of 10 - 15 filaments each on an average, expressed as percentage and recorded on 8th day after inoculation and on termination of experiment, i.e., 19th day.

The estimation of spore germination and growth were made microscopically by

observing the behaviour of increasing cell number/filaments, spores and young germling on agar plates. Sporulation frequency was estimated by observing one colony in terms of vegetative cells and spores represented on an average of 10 observations on random basis in triplicate cultures.

Ammonical nitrogen uptake was measured in an orion ion meter using ammonia electrode in cultures which were supplemented with 0.2 M of ammonical nitrogen source in the form of ammonium sulphate and subjected to normal growth period of 18 days. Data represented here indicate mean of three observations.

Results and Discussion

Anabaena doliolum and *Nostoc muscorum* were subjected to growth in basal medium containing 0.0 M to 0.60 M of sucrose as organic carbon source. It was observed in case of *A. doliolum* that although growth was supported in media containing sucrose upto 0.60 M but there was evidently a gradual decline in all the growth parameters studied in comparison to control which exhibited best growth. Cyanobacteria being obligate photoautotrophic, hence, synthesis of chl-a as well as protein is inhibited in presence of sucrose in the medium and at 0.60 M of sucrose there was no measurable growth as the cultures were subjected to osmotic stress (Table 1). Absorption spectra of acetone soluble pigments support the view that carotenoid as well as chlorophyll synthesis of sucrose supplemented cyanobacterial cultures was reduced (Fig.1).

In case of *N. muscorum* although measurable growth was detected upto 0.75 M of sucrose but measurable protein content recorded only upto 0.6 M of sucrose as it is in *A. doliolum* and cultures show declining pattern of growth in sucrose containing basal medium in comparison to control. There were also linear changes in pH and conductivity values in *A. doliolum*. However, appreciable changes in pH and conductivity values were reflected suggestive of interaction among nutritive ions and sucrose molecules present in the medium; where cyanobacteria growing (Table 2), absorption spectra recorded do not show much reduction in growth in terms of chlorophyll and carotenoid pigments upto 0.2 M but thereafter the decline is sharp and interference in photosynthetic process is evident (Fig. 2).

Percentage heterocyst frequency in both the cyanobacteria does show an increase upto 0.15 M (beyond it decrease) indicating the effect of these at level of cellular differentiation by producing long filaments and poorly developed heterocyst but more in number having thin wall and less conspicuous polar bodies. At higher concentrations of sucrose a short lag phase is observed, followed by loss of pigments but heterocyst frequency increased to some extent so much so that nitrogen fixation is affected, i.e., it does not increase at higher concentrations of sucrose. Although heterocyst frequency was more indicative of the fact that sucrose being organic carbon source, interferes with normal growth as well as fixation of dinitrogen in cyanobacteria studied. Permeability of cell membrane is also altered in *A. doliolum* and *N. muscorum;* when combinations of sucrose and ammonium source were supplemented into the medium and ammonical nitrogen uptake was studied. It was found that sucrose increased uptake of NH_4^+ ion from the medium (Table 3-5).

Assimilation of sucrose was observed from the point of view of differential

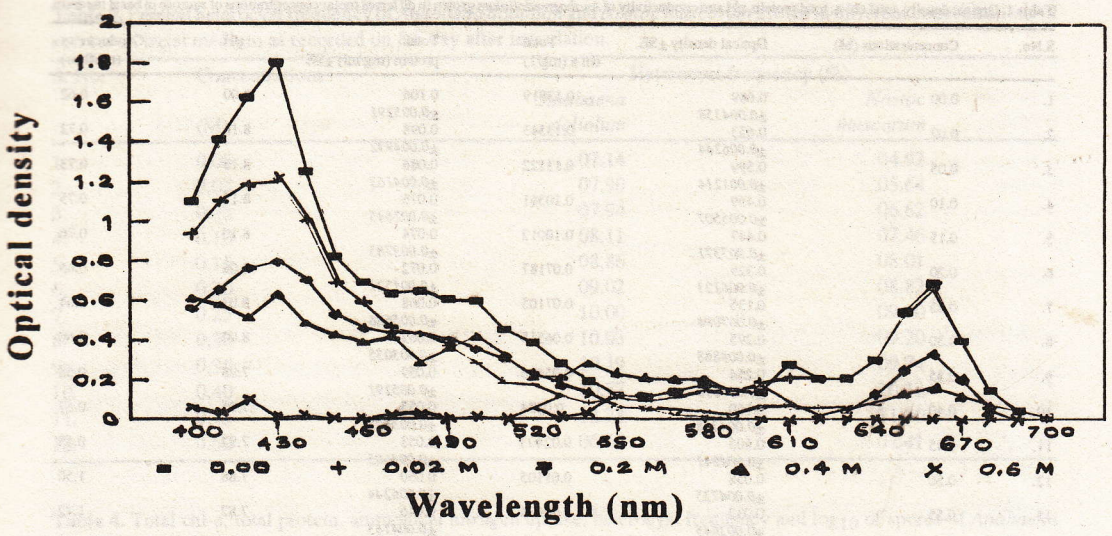

Fig.1. Absorption spectra of acetone-soluble pigments of *Anabaena doliolum* grown in various concentrations of sucrose into basal medium.

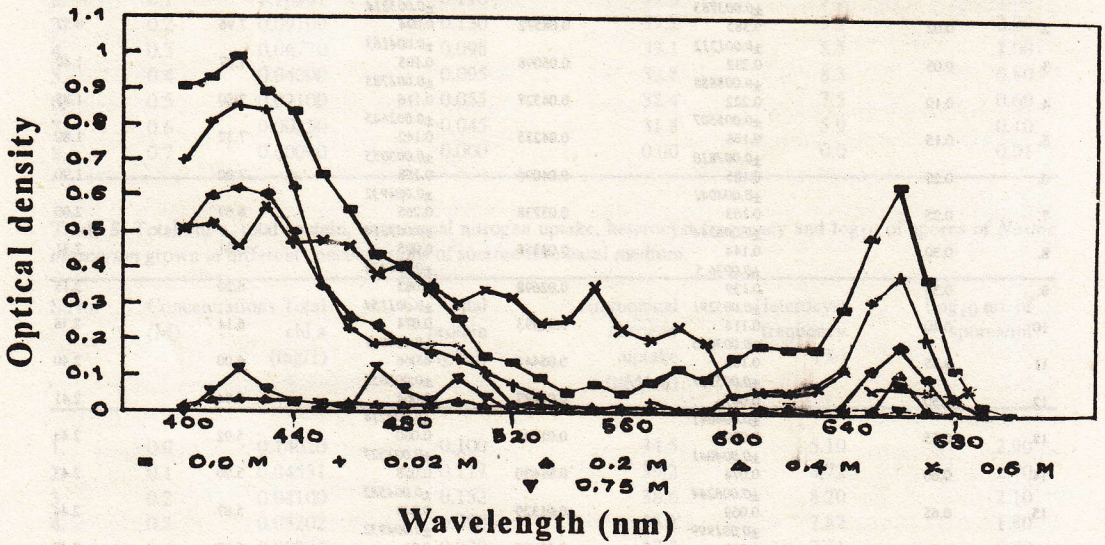

Fig. 2 Absorption spectra of acetone-soluble pigments of *Nostoc muscorum* grown in various concentrations of sucrose into basal medium.

Table 1. Optical density, total chl-a, total protein, pH and conductivity of *Anabaena doliolum* grown in different molar concentrations of sucrose in basal medium.

S.No.	Concentrations (M)	Optical density ±SE	Total chl a (mg/l)	Total protein (mg/ml) ±SE	pH	Conductivity (mMhos)
1.	0.00	0.689 *±0.004358*	0.13819	0.106 *±0.005291*	8.00	0.62
2.	0.02	0.653 *±0.006244*	0.13543	0.098 *±0.004932*	8.16	0.72
3.	0.05	0.599 *±0.001214*	0.13222	0.086 *±0.004163*	8.15	0.73
4.	0.10	0.459 *±0.005507*	0.10361	0.076 *±0.002645*	8.12	0.75
5.	0.15	0.447 *±0.007371*	0.10012	0.074 *±0.003785*	8.10	0.76
6.	0.20	0.329 *±0.006123*	0.07187	0.072 *±0.001527*	8.06	0.65
7.	0.25	0.135 *±0.007094*	0.07105	0.068 *±0.005686*	8.03	0.64
8.	0.30	0.295 *±0.008888*	0.06515	0.062 *±0.003055*	8.00	0.60
9.	0.35	0.254 *±0.006110*	0.05426	0.059 *±0.005291*	7.98	0.58
10.	0.40	0.160 *±0.004358*	0.0774	0.055 *±0.003605*	7.95	0.57
11.	0.45	0.105 *±0.004041*	0.02403	0.053 *±0.003605*	7.92	0.55
12.	0.50	0.058 *±0.004725*	0.01105	0.050 *±0.006244*	7.88	1.50
13.	0.55	0.032 *±0.002645*	0.00634	0.046 *±0.004163*	7.82	1.42
14.	0.60	0.008 *±0.001154*	0.00193	0.000 *±0.000000*	7.79	1.40

Protein = 0.0379308 + 0.1007456 OD (r = 0.8948045 **)

* ± SE represented as italic figures as given below each observation.

Table 2. Optical density, total chl-a, total protein, pH and conductivity of *Nostoc muscorum* grown in different molar concentrations of sucrose in basal medium.

S.No.	Concentrations (M)	Optical density ±SE	Total chl a (mg/l)	Total protein (mg/ml) ±SE	pH	Conductivity (mMhos)
1.	0.00	0.635 *±0.003785*	0.14196	0.090 *±0.003214*	8.00	0.65
2.	0.02	0.385 *±0.001212*	0.08572	0.094 *±0.004163*	7.96	0.57
3.	0.05	0.232 *±0.008888*	0.05098	0.105 *±0.003785*	7.67	1.40
4.	0.10	0.222 *±0.005507*	0.04327	0.116 *±0.002645*	7.50	1.85
5.	0.15	0.196 *±0.007810*	0.04235	0.142 *±0.003055*	7.32	1.80
6.	0.20	0.185 *±0.004041*	0.04096	0.158 *±0.004932*	7.00	1.90
7.	0.25	0.163 *±0.006244*	0.03738	0.265 *±0.003214*	6.69	2.00
8.	0.30	0.144 *±0.0036.5*	0.03358	0.095 *±0.004358*	6.31	2.31
9.	0.35	0.139 *±0.005291*	0.02698	0.082 *±0.001154*	6.20	2.15
10.	0.40	0.114 *±0.003055*	0.02593	0.074 *±0.004041*	6.14	2.38
11.	0.45	0.105 *±0.005127*	0.02441	0.066 *±0.005033*	6.00	2.40
12.	0.50	0.087 *±0.004041*	0.01887	0.064 *±0.003214*	5.94	2.41
13.	0.55	0.079 *±0.004041*	0.01744	0.060 *±0.001527*	5.92	2.43
14.	0.60	0.074 *±0.006244*	0.01630	0.058 *±0.004582*	5.90	2.43
15.	0.65	0.069 *±0.001999*	0.01329	0.049 *±0.004932*	5.87	2.44
16.	0.70	0.028 *±0.004358*	0.00656	0.21 *±0.003214*	5.80	2.48
17.	0.75	0.12 *±0.001732*	0.00265	0.011 *±0.002081*	5.72	2.49
18.	0.80	0.000 *±0.000000*	0.0000	0.00 *±0.000000*	5.70	2.50

* ±SE represented as italic figures as given below each observation.

Table 3. Percent heterocyst frequency of *Anabaena doliolum* and *Nostoc muscorum* grown in different concentrations of sucrose in basal medium as recorded on 8th day after inoculation.

S. No.	Concentrations (M)	Heterocyst frequency (%) *Anabaena doliolum*	*Nostoc muscorum*
1.	0.00	07.14	04.92
2.	0.02	07.90	05.64
3.	0.05	07.94	06.62
4.	0.10	08.11	07.46
5.	0.15	08.86	08.01
6.	0.20	09.02	08.82
7.	0.25	10.00	09.00
8.	0.30	10.03	09.20
9.	0.35	10.19	09.74
10.	0.40	10.77	10.12
11.	0.45	12.28	11.80
12.	0.50	06.62	07.41

Table 4. Total chl-a, total protein, ammonical nitrogen uptake, heterocyst frequency and $\log_{10}$ of spores of *Anabaena doliolum* grown in different concentrations of sucrose into basal medium.

S. No.	Concentrations (M)	Total chl a (mg/l)	Total protein (mg/ml)	Ammonical nitrogen uptake (μM/ml)	Heterocyst frequency (%)	$\log_{10}$ no. of spores/ml
1.	0.0	0.14001	0.103	32.0	5.6	3.50
2.	0.1	0.10391	0.116	39.0	9.5	3.30
3.	0.2	0.09100	0.130	37.2	9.1	2.20
4.	0.3	0.06770	0.098	33.1	8.5	1.00
5.	0.4	0.04000	0.095	32.8	8.3	0.80
6.	0.5	0.02100	0.055	32.4	7.5	0.60
7.	0.6	0.00250	0.045	31.8	5.9	0.10
8.	0.7	0.00000	0.000	0.00	0.0	0.01

Table 5. Total chl-a, total protein, ammonical nitrogen uptake, heterocyst frequency and $\log_{10}$ of spores of *Nostoc muscorum* grown in different concentrations of sucrose into basal medium.

S.No.	Concentrations (M)	Total chl a (mg/1)	Total protein (mg/ml)	Ammonical nitrogen uptake (μM/ml)	Heterocyst frequency (%)	$\log_{10}$ no. of spores/ml
1.	0.0	0.14020	0.100	34.5	5.10	2.90
2.	0.1	0.04531	0.117	39.2	8.72	2.70
3.	0.2	0.04100	0.152	38.6	8.20	2.10
4.	0.3	0.03202	0.090	38.2	7.82	1.80
5.	0.4	0.00270	0.070	37.3	7.51	0.70
6.	0.5	0.01900	0.060	36.8	6.85	0.50
7.	0.6	0.01701	0.030	36.0	5.20	0.20
8.	0.7	0.00000	0.000	00.0	0.00	0.05

Table 6. Analysis of variance of regression lines of OD on protein of *Anabaena doliolum* and *Nostoc muscorum*.

Anabaena doliolum				*Nostoc muscorum*			
Source of variation	DF	MSS	F	Source of variation	DF	MSS	F
Regression	1	0.0069602	48.2008	Regression	1	NS	NS
Residual	12	0.0001444		Residual	15	NS	

effect on both the cyanobacteria but exact nature of inhibition is yet to be understood. When cyanobacteria are subjected to salinity-stress carbohydrates accumulated into the cells at higher temperature (35° C) as an osmoticum. Statistical analysis has been given in Table-6.

The heterotrophic potential of any autotrophic organism is established by simple growth experiments under conditions where photoautotrophic route is blocked and the organism is compelled to utilize the exogenously supplied carbon sources. Failure of growth stimulation in light by some other carbon source may be due to the reason that these compounds are not metabolized because of the absence of required enzyme.

The concentration dependent stimulation of growth in glucose may be due to a low rate of glucose entry into cells. It seems probable that higher levels of glucose result in an increase in the rate of assimilation thereby increasing ammonical nitrogen uptake. Nitrogen fixation and chlorophyll content are inversely related under photoautotrophic conditions. Loss of chlorophyll at different concentrations of glucose may cause some decline in oxygen evolution and create micro-aerobic environment and may establish favourable conditions for nitrogen fixation[8]. It was suggested that operation of HMP pathway in glucose metabolism, which may only function in the dark enabling the cell to utilize photosynthetically assimilated carbon reserves[1], and in heterocysts, which do not assimilate CO_2 photosynthetically, but, receive reduced carbon compounds from vegetative cells[9]. The increased activities of G-6-P dehydrogenase, 6-P-gluconate dehydrogenase in heterocysts[10]. Such compartmentations implies that under photoautotrophic N_2 - fixing conditions heterocysts are carbon limited, and have metabolic consequenses.

It was reported that cyanobacteria thrive at elevated salinities by accumulating low molecular weight carbohdrates as internal osmotica in response to external osmotic stress[11] and few studies[12] have dealt specifically with organic solutes accumulated by cyanobacteria adapting to salt stress.

For these compounds to be tolerated at high intracellular concentrations they must act as compatible solutes, i.e., they must allow enzymic function to continue at reduced water activity. There is general trend toward the accumulation of sucrose or trehalose by osmotically stressed stenohaline fresh water isolates. Fresh water species *Nostoc muscorum* PCC 71119 accumulated sucrose[13]. Thus, it appears that organic compounds are important osmoregulatory solutes in a large number of cyanobacteria. A direct relationship has been

observed between osmotic conditions and the temperature dependence of sucrose synthesis. Warr *et al.*[14,15] have reported effects of NaCl and KCl together with either sucrose or glycine betaine respectively on the activity of glutamine synthetase, a key enzyme of nitrogen metabolism.

Osmoregulatory solutes which accumulate in high concentrations in cyanobacteria and in doing so do not inhibit enzyme activity have been termed compatible solutes. These compatible solutes are thought to act either by cooperative binding to protein which does not induce a conformational change, or by affecting the water domain surrounding the hydrophobic groups in protein rather than binding to the protein itself. None of the osmoregulatory solutes found among the cyanobacteria have been assayed for their effect on enzyme activity in cell-free cyanobacterial systems, but, some have been assayed for their effect on enzyme activity in cell-free systems. Monosaccharides and disaccharides are considered poor compatible solutes because the high concentration inhibit enzyme activity[17,18]. This may account, at least in part, for the poor salt-tolerance of fresh water cyanobacteria. Glycine betaine is well documented as a good compatible solute, when present at 0.5 M it does not significantly inhibit enzyme activity, mitochondrial or chloroplast function, polysomes or protein synthesis and may even partly protect some enzymes against salt-toxicity[19].

Result of present experiment confirm earlier findings that none of the developmental process including spore germination, vegetative growth and sporulation of *A. doliolum* and *N. muscorum* occur heterotrophically. On the contrary all the process listed above are light dependent and that light is indeed involved in the control of some developmental characters in heterocystous cyanobacteria.

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