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Growth and Protein Profiling by the Cyanobacterium *Anacystis nidulans* Strains at Different Temperature and Photoperiod

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Abstract

The *Anacystis nidulans*, a prokaryotic, oxygen-evolving, photosynthetic Gram-negative bacterium was isolated from the Sambhar Lake (26.94561 Latitude and 75.20968 Longitude), Jaipur, Rajasthan (India) and cultures were maintained in BG 11 medium and kept at the temperature of 25 ± 2°C, illuminated with white fluorescent lamps at a light intensity of 2,500 lux with photoperiod of 12 hours light/dark cycle and aerated continuously. The effects of photoperiod and temperature on growth and protein expression by *Anacystis nidulans* strains were studied under laboratory conditions. To find out the best culture condition, *Anacystis nidulans* was cultivated in BG 11 medium under various combinations of light duration and temperature (ALR, ALC, CLR, CLC, and NDL). The effect of these treatments on growth was measured by optical density, cell count, and dry weight. Protein content was measured quantitatively by Bradford assay. The variation in the protein expression in different culture conditions was measured using SDS-PAGE. The densitometric analysis was also carried out from the SDS-PAGE for the determination of increase or decrease protein expression level. Maximum growth and protein were observed in ALR condition.

Keywords: Anacystis nidulans; Bradford assay; Densitometer; Photoperiod; SDS-PAGE

Abbreviations: ALR: Alternate Light at Room Temperature (27-36°C); ALC: Alternate Light in Culture Chamber (20-25°C); CLR: Continuous Light at Room Temperature (27-36°C); CLC: Continuous Light in Culture Chamber (20-25°C); NDL: Natural Day Light at Room Temperature (27-36°C)

Introduction

Anacystis nidulans was isolated from a shallow Sambhar lake, Jaipur, (Rajasthan), where it occurred in massive amounts. The culture was rendered free from contaminant algae and other cyanobacteria. Anacystis nidulans a prokaryotic, oxygen-evolving, photosynthetic Gram-negative bacteria, survive in a wide variety of extreme environmental conditions [1]. The growth kinetics and physiological properties of phototrophic organisms are markedly influenced by the length of the photoperiod and by the ratio of light to dark hours [2-6]. The importance of the photoperiod has been demonstrated by photosynthesis i.e. a process which delivers energy for carbon assimilation in the light and nitrogen assimilation in the light and in the dark [6-8]. The synthesis of various cellular components is affected by the presence or absence of light-irradiance. In continuous cultures it was found that the total of DNA, RNA and proteins increased at an apparent constant rate during a light-dark cycle [6]. Changes in light regimes and temperatures have been shown to bring about differences in pigment and biochemical composition of microalgae [9-12]. The light/dark photoperiod may be more beneficial than other regimes, as cell number is sustained in exponential phase longer [13]. Experiments suggest that exposure to various light treatments results in a qualitative and quantitative regulation of individual proteins in Synechococcus [14]. Similarly another report showed that the cellular content of nucleic acids and protein decreased during light periods and increased during dark periods in *Synechococcus sp*. strain PCC 6301 [15].

The expression of the total proteins varying with response to different temperatures and photoperiods can be determined qualitatively as well as quantitatively. Quantification of microalgal biomass Protein content is carried out either by colorimetric methods [16-18] or by methods that measure the concentration of elemental nitrogen. [19,20]. Hydrolytic enzymes and chemicals such as sodium dodecyl sulfate (SDS) can be used to lyse the cell walls [21].

In this experiment algal cultures were exposed to different temperature, L/D cycles, and constant light, so a characteristic set of cellular and metabolic response was triggered. These culture conditions are responsible for changing in growth pattern and protein profiling of Anacystis nidulans. The growth was measured by optical density, cell count and dry weight. Protein content was estimated quantitatively by Bradford assay (1976) and the changes in protein expression can be detected through changes in polypeptide patterns observed on SDS-PAGE. These polypeptides were expresses in different quantity in different culture conditions i.e. measured by densitometric techniques.

Materials and Methods

Organism

The experimental organism Anacystis nidulans was isolated from Sambhar Lake, Jaipur (Rajasthan). The cells were grown in modified BG11 medium. Culture temperature was maintained at 25°C to 30°C; pH was 9.0. To remove inhibitory substances and curb any possible mineral deficiency, time to time the entire growth medium was replaced with fresh medium. Experiment to evaluate the impact of different photoperiod and temperature on Anacystis nidulans were carried out in departmental laboratory.

Culture conditions

In order to find out impact of different culture conditions, cultures

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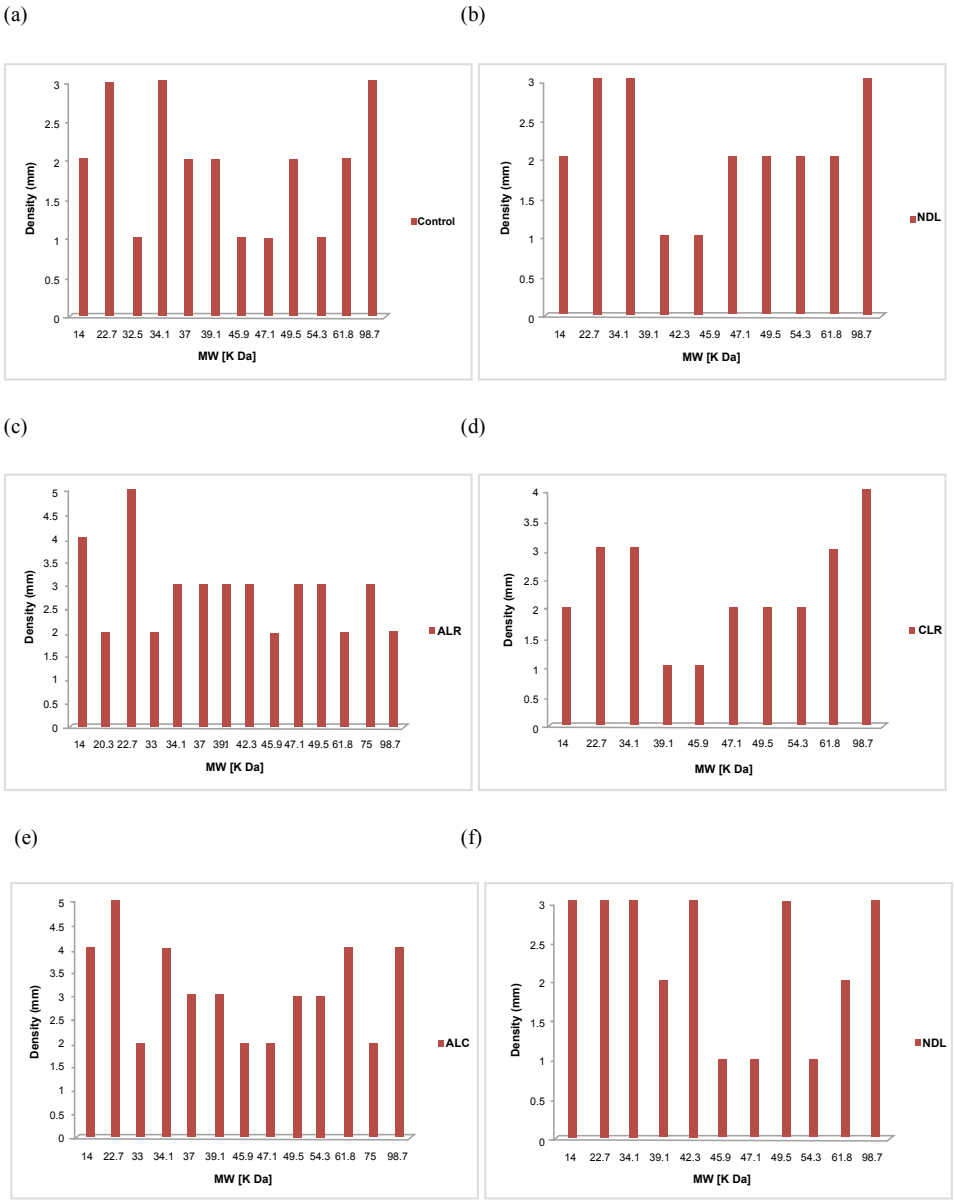
were subjected to five different combinations of temperature range and illumination, the following conditions were tried for present study:

1. Series receiving alternate light and dark period (12:12) at 27-36°C. (ALR)
2. Series receiving alternate light and dark period (12:12) at 20-25°C. (ALC)
3. Series receiving constant light at 27-36°C. (CLR)
4. Series receiving constant light at 20-25°C. (CLC)
5. Series receiving natural day and dark period at North facing window 27-36°C.(NDL)

Three test tube sets containing 10 ml of BG11 medium and 2 ml of freshly growing culture were subjected to different culture conditions and there growth was determined through optical density (OD), Dry weight (DW) and cell count (CC). Simultaneously conical flasks containing 250 ml Zarrouk's medium and 50 ml Anacystis nidulans cultures were subjected with each of these culture conditions for protein estimation (Figure 1).



ALR: Alternate Light at Temperature 27-36°C
ALC: Alternate Light in Culture Chamber (20-25°C)
CLR: Continuous Light at Temperature 27-36°C
CLC: Continuous Light in Culture Chamber (20-25°C)
NDL: Natural Day Light at Temperature 27-36°C

Figure 1: Densitometric Analysis of SDS-PAGE banding pattern in *Anacystis nidulans*.

Growth measurement parameters

Optical density, cell count and dry weight: Observations were carried out over a period of five weeks after initial readings. Growth was measured through Optical density, cell count and dry weight. Optical density was recorded by using colorimeter at 670 nm and cell count examination was performed using a compound microscope (Nikon SE, Japan). Cells of Anacystis nidulans were counted in at least 10 slides from each sample using a homogeneous culture suspension. Five flasks with 50-50 ml media were taken and inoculated Anacystis nidulans at 0.1 Optical Density. Dry weight determination was done by using 50 ml algal sample of suspension which was filtered through a Whatman GF/C Filter of 47 mm diameter. The filter was dried in an oven for overnight at 70°C, then in dessicator for 20 min for cooling and weighed. Cultures were shaken gently thrice a day to avoid clumping and accelerate the growth process.

Protein profiling

The effect of temperature and photoperiod on protein profile was determined through SDS-PAGE. Soluble proteins were extracted by homogenizing plant tissues with buffer containing (0.5M Tris-HCl, 8 M Urea, 5% (w/v) SDS, 20% (v/v) Glycerol and 10% (v/v) β-Mercaptoethanol; final pH 6.8) and centrifuged at 4°C for 20 min at 10,000 rpm. The supernatant was used as crude protein extract and protein quantity was measured following Bradford assay. Crude protein extracts from all treatments were resolved on 12% SDS-polyacrylamide gel [22] and stained with 0.1% Coomassie Brilliant Blue (R250) dye. In order to score and preserve banding pattern, the gel was subjected to image scanning using BIO-RAD GS –700 Imaging Densitometer (USA) and the protein profiles were obtained for each variety. The bands were designated on the basis of their molecular weight, for this purpose molecular weight marker ranging from 14.4 kDa to 116.0 kDa was loaded simultaneously with samples. The distance run by amplified fragment, from the well was translated to molecular weight with reference to protein molecular weight marker. The presence of each band was scored as (+) plus and it absence as minus (-) (Table 1).

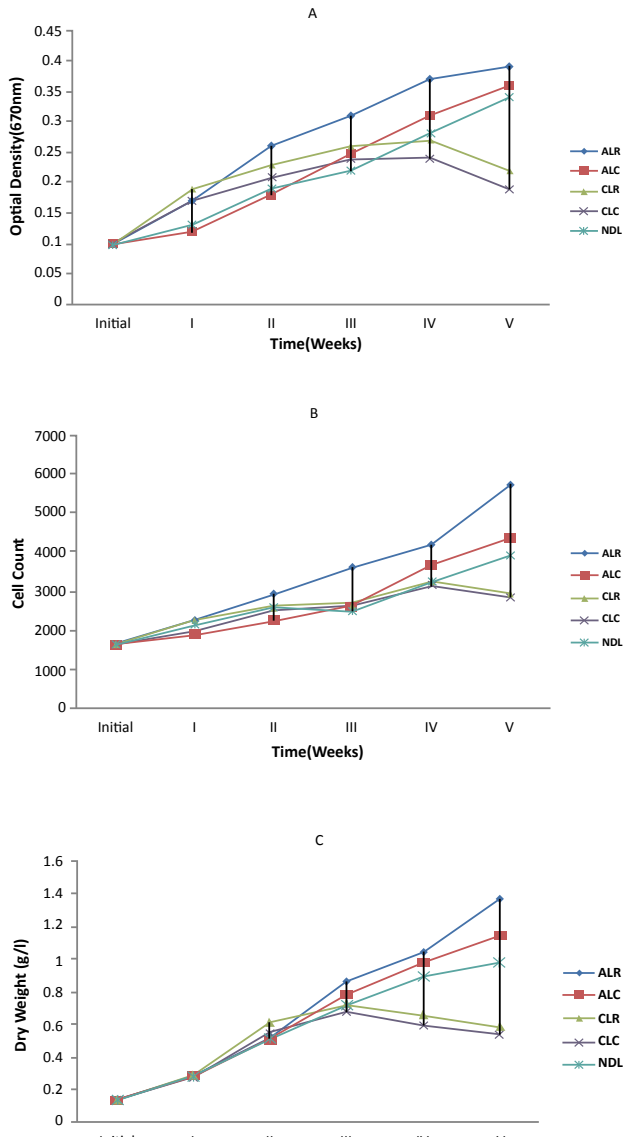
MW [KDa]	C	ALR	ALC	CLR	CLC	NDL
14	+	+	+	+	+	+
20.3	-	+	-	-	-	-
22.7	+	+	+	+	+	+
32.5	+	-	-	-	-	-
33	-	+	+	-	-	-
34.1	+	+	+	+	+	+
37	+	+	+	-	-	-
39.1	+	+	+	+	+	+
42.3	-	+	-	-	-	+
45.9	+	+	+	+	+	+
47.1	+	+	+	+	+	+
49.5	+	+	+	+	+	+
54.3	+	-	+	+	+	+
61.8	+	+	+	+	+	+
75	-	+	+	-	-	-
98.7	+	+	+	+	+	+

C- Control
ALR- Alternate Light at Temperature 27-36°C,
ALC- Alternate Light in Culture Chamber (20-25°C),
CLR- Continuous Light at Temperature 27-36°C,
CLC- Continuous Light in Culture Chamber (20-25°C),
NDL- Natural Day Light at Temperature 27-36°C.

Table 1: SDS-PAGE banding pattern presence and absence (+/-) of Anacystis nidulans.

Results and Discussion

The results presented in this paper have shown that the different culture conditions induce different cellular and metabolic activities i.e. responsible for different growth pattern and variation in protein expression. Among all of these conditions, alternate light and dark period at 27-36°C (ALR) shows maximum growth and followed by alternate light and dark period at 20-25°C (ALC), natural day light at 27-36°C (NDL), constant light at 27-36°C (CLR) and minimum growth observed in constant light at 20-25 °C (CLC). In alternate light and dark 12:l2 in both the sets of temperature i.e. 27-36°C and 20-25°C growth was higher. The growth at alternate light and dark period at 27-36°C (ALR) was more intense than in the other four culture conditions. The difference became more evident after 28 days, where cell concentration



ALR: Alternate Light at Temperature 27-36°C; ALC: Alternate Light in Culture Chamber (20-25°C); CLR: Continuous Light at Temperature 27-36°C; CLC: Continuous Light in Culture Chamber (20-25°C); NDL: Natural Day Light at Temperature 27-36°C

Figure 2: Effect of different culture conditions on growth parameters (A) optical density (B) Cell count (C) Dry weight of *Anacystis nidulans*.

reached 2.56 times of the initial record i.e. higher than that observed in other four sets. After 35 days of the initial record the OD was enhanced 3.9 times at 27-36°C (ALR) and 20-25°C (ALC) OD was only 3.6 times. Cell counts were also support our results, higher number of cells was scored at 27-36°C (ALR) which showed an increase of about 3.5 times the initial number, On the other hand at 20-25°C (ALC) the number of cells were increased only about 2.68 times. The biomass quantity measurement also favours the OD and C.C. results i.e. increased 10.5 times (ALR) and 8.84 times (ALC) of the initial record (Figure 2a, 2b, 2c). The growth also increased in natural day light at 27-36°C (NDL) up to fifth week i.e. OD (3.4 times), CC (2.4 times), and D.W. (7.6 times) of the initial record. Earlier reports of [23-25] confirm that alternate L/D period and 30°C to 35°C temperature is the optimal culture condition for cyanobacterial cellular and metabolic activity [26,27] experiment previously proved that under constant illumination the algae can perform only a reduced growth and the alternate L/D cycles may induces the growth and different metabolic pathways. Under continuous illumination out of the two sets of temperature i.e. 27-36°C (CLR) and 20-25°C (CLC) condition algae grows rapidly up to third week but after third week the growth was gradually decreased and algal cultures were degraded probably due to photo inhibition. Jensen and Knutsen and Vonshak demonstrated that use of high light intensity in some algal cultivation can lead to photo inhibition [28,29]. The photo inhibition is caused by photo oxidation reaction inside the cell due to excess light that cannot be absorbed by the photosynthetic apparatus [12]. Under CLR condition O.D., C.C., and D.W. increased only 2.2 times, 1.8 times and 4.5 times of the initial record. Similarly growth in the (CLC) condition was increased by only O.D. (1.9 times) C.C. (1.7 times) and D.W. (4.16 times) of the initial recorded data. In this way CLR and CLC condition shows reduced growth probably due to the photo inhibition i.e. caused by photo oxidation reaction [28,29].

The quantitative estimation of the extracted proteins was determined by the Bradford assay due to its simplicity, accuracy and relative insensitivity to interfering compounds [30]. This method requires comparing the spectrophotometric absorbance of the unknown sample at a wavelength of 595 nm to a calibration curve prepared using standard solutions, Bovine serum albumin (BSA) of a protein. The Bradford results shows that maximum protein content was present in ALR condition i.e. 4.98 mg/ml that is gradually decreased in the ALC (4.42 mg/ml), NDL (4.18 mg/ml), CLR (3.45 mg/ml) and minimum in the CLC (3.29 mg/ml) condition compare to the control. Relatively few reports regarding quantification of cyanobacterial proteins by Bradford assay can be found in the literature [31,32].

The photoperiod and temperature affect on cellular and metabolic activities of the organism that lead to increase or decrease the level of protein expression. Extracted proteins from algal culture grown at different photoperiod and temperature were subjected to 12% polyacrylamide gel electrophoresis along with PMW-B for estimation qualitatively. Total 14 polypeptide species were observed in SDS-PAGE profile of control initial culture (Figure 3) and the molecular weight of these polypeptides were ranging from 14.0 kDa to 98.7 kDa. The similar polypeptide bands in each culture condition were observed around 22.7 KDa, 33.0 KDa, 45.9 KDa, 47.1 KDa, 49.5 KDa, 61.8 KDa, 75 KDa and 98.7 KDa. Comparison of all culture conditions (ALR, ALC, NDL, CLR and CLC) samples with control sample indicated increased thickening and sharpening of bands. The present study found that Anacystis nidulans showed 4 basic differences in terms of protein content under different culture conditions i.e.

1. Induce production of some new proteins that not present in the normal cultures;
2. Inhibition of some proteins that are produced by the normal cultures;
3. Increase in the level of expression of some proteins;
4. Decrease in the level of expression of some proteins that are present in the normal cultures.

Weber and Jung [33] demonstrated that changes in protein profiling and newly formed proteins might be helping cyanobacteria to tolerate adverse stress conditions. The electrophoresis results shows that alternate light and dark period at 27-36°C (ALR) was the optimum condition for the expression of many polypeptides compare to other culture conditions. Two peptides (37.0 KDa and 42.3 KDa) were present all the samples of alternate L/D cycles at both temperatures, while absent in the constant light treated samples. The genes of these polypeptide expressions were inhibited by the excess light condition due to probably photo inhibition and photo-oxidation. [29,12]. Two other novel peptides of 75 KDa and 33 KDa was express in ALR and ALC that were similar in photoperiod and light intensity but differ in temperature. The genotypes of these peptides also inhibited in the constant light condition due to probably photo inhibition or photo oxidation. The genotype of 20.3 KDa peptide was uniquely expressed only in the alternate light and dark period at 27-36°C condition. Another novel band of 42.3 KDa was observed in both ALR and NDL conditions that are approximately same in the temperature and differ in light intensity. The 54.3 KDa band almost degraded in the genotype at alternate light and dark period at 27-36°C while consistently present in other four conditions.

The increase or decrease in the level of protein expression is depends on the cellular and metabolic activities of the organism i.e. affected by availability of light and different temperatures. In the optimum condition many proteins were over expressed and in adverse condition some peptides expression was inhibited. In order to score and preserve banding pattern, the gel was scanned under BIO-RAD GS-700 Imaging Densitometer. The density of band shows the quantity of particular peptide means highly dense band represent that this peptide was expressed in large amount and light band shows low level expression. The 14.0 KDa and 22.7 KDa peptides expression was 5mm in the all L/D cycle conditions due to its high metabolic activity

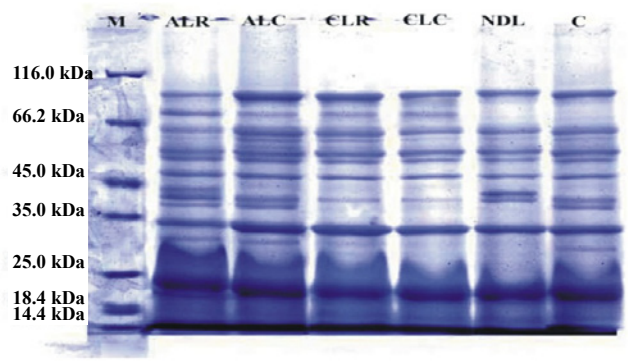


Figure 3: Coomassie blue-stained polypeptide profiles of extracted protein separated by SDS-PAGE. The proteins were extracted from *Anacystis nidulans* cells grown under different conditions i.e. ALR- Alternate Light/Dark cycle at Temperature 27-36°C, ALC- Alternate Light/Dark cycle in Culture Chamber (20-25°C), CLR- Continuous Light at Temperature 27-36°C, CLC- Continuous Light in Culture Chamber (20-25°C), NDL- Natural Day Light (27-36°C).

(Figure 1c,1e) whereas in the constant light the expression was only 3 mm (Figure 1b,1d). Two peptide bands 37.0 KDa and 42.3 KDa were absent in the initial sample and their expression also inhibited in the constant light but in the favourable condition (alternate light and dark period) these peptides were expressed approximate 3 mm level (Figure 1c,1e,1f). A novel 45.9 KDa peptide was expressed in large amount (3 mm) at alternate light and dark period at both temperatures; while in constant light it expressed only 2 mm level. Two peptides of 33 KDa and 75.0 KDa proteins were uniquely expressed up to 3 mm level in the alternate light and dark period both set of temperature (Figure 1c,1e). The expression of 39.1 KDa peptide was 3 mm in the alternate light and dark period while in constant light it was expressed only 1mm (Figure 1b,1d). All of these results tend to agree with [34] who reported that the production of novel proteins or the increased production of already existing proteins, which are only produced under stress conditions due to stress response (Figure 1).

Conclusion

Experiments were conducted with a view to determining the deleterious and differential effects of temperature and photoperiod on the growth and protein profiling of Anacystis nidulans. In conclusion our proteomic study of the differential protein profiles under different environmental conditions found that Anacystis nidulans showed both constancy of protein profiles and variability of the relative abundance of some abundant proteins under different culture conditions. The effect of both temperatures (27-36°C and 20-25°C) are probably responsible for the increase or decrease the level of protein expression whereas effect of alternate light and continuous light induces or suppresses the expression of some peptide expression. Some novel protein will express and some exiting peptide expression will be suppressed. In continuous light algal growth may be reduces due to photo inhibition and photo oxidation.

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