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**Original Research Article**

# Effect of light and temperature on photosynthetic oxygen evolution in *Mastigocladus laminosus*

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**A B S T R A C T**

Temperature responses of photosynthesis of Matigocladus laminosus (isolated from hot water spring Tattapani, HP) was determined in terms of photo evolution of oxygen using cells grown at 45$^{o}$C. $O_2$ evolution from uniformly fragmented cells was tested at 25$^{0}$C,35$^{0}$C.45$^{0}$C and 50$^{o}$C. The maximum $O_2$ evolved (2, µ moles $O_2$/mg protein/5 min) at 45$^{o}$C, indicating the highest photosynthetic activity of this thermopile at test temperature of 45$^{o}$C similar to the habitat temperature. In a common test temperature of 45$^{o}$C when photo evolution of oxygen was compared using cells adapted both at 45$^{o}$C and 25$^{o}$C, it was observed that index of photosynthetic adaptation was higher (8.8) for 25$^{o}$C compared to 45$^{o}$C grown cells (1.2). RUBP Carboxylase maximum activity detected at 45$^{o}$C (approx. 72 pmoles of $CO_2$ fixed/ug chl-a/min) indicated the presence of $C_3$ mode of carbon fixation under thermal habitat shows that RuBP Case in this organism has high affinity towards elevated temperature which is a prerequisite factor for thermal adaptation for photoautotrophs.The study is useful to understanding of photosynthesis oxygen generation in respose to light and temperature as well as opening up debate about its evolutionary origin, use for biophotolysis and for generation of biofuels and distribution pattern in hot water spring.

## Introduction

Cyanobacteria are among the very few groups that can perform oxygenic photosynthesis and respiration simultaneously in the same compartment, and some cyanobacterial species are able to fix nitrogen. This combination of metabolic pathways is unusual and this metabolic flexibility may be responsible for the evolutionary hardiness of the cyanobacteria and their ability to thrive under a wide range of conditions (Vermaas, 2001). The thermal cyanobacteria have developed adaptive strategies so that they can withstand and grow under the sub-optimal temperature regime of their native habitats.

Cyanobacteria adjust their photosynthetic apparatus in order to acclimate to the prevailing temperature.They tend to have decreased photosynthetic capacity (Pmax) at low temperatures due to depressed activity of ribulose-1,5-bisphosphate carboxylase (Rubisco) (Li et al ., 1984; Raven & Geider, 1988).

The effect of temperature on biochemical reactions makes it one of the most important environmental factors and its effects include: the rate of photosynthesis (Davison, 1991; Ensminger et al.2001), affinity of ribisco for $CO_2$; increased rate of respiration at high temperature; increased thermal dissipation of excess absorbed energy; non-linear relationships of $O_2$ evolution; and chlorophyll fluorescence parameters. In addition, temperature regime influences latitude, altitude and drainage basin distribution, as well as seasonality of freshwater benthic algae (Sheath 1984; DeNicola 1996).

The light-induced oxidation of water by Photosystem II (PS II) of higher plants, algae, and cyanobacteria, is the main source of atmospheric oxygen(Shinkarev, 2003 ; Barber, 2012) Photosystem II (PSII) is a light-dependent water: plastoquinone-oxidoreductase that uses light energy to oxidize water and to reduce plastoquinone (reviewed in Ke, 2001; Renger, 2001) . PSII is a multisubunit protein complex located in the thylakoid membranes of all types of plants, algae, and cyanobacteria (Barber, 2003; Diner & Babcock 1996; Wydrzynski & Satoh 2005)) .$2H_2O+2PQ \rightarrow O_2+2PQH_2$, is the overall reaction driven by PSII, where PQ and $POH_2$ are oxidized and reduced

High temperature and low combined nitrogen source in the hot water springs, favour the growth of $N_2$ fixing organisms including cyanbacteria (Wards and Castenholz, 2000). Cyanobacteria has the ability to capture light at low intensities and at a range of wavelengths (Litchman et al., 2010 ) give the ability ability togrow in warmer temperatures. Mastigocladus species are known to be copmponent of algal-bacterial mats in neutral to alkaline hot water spring (Castenholz, 1976,1977;Fagerberg & Arnott,1979), being capable of cell division and growth at temperature ranging from $50^0$C to $64^0$C (Holton,1962;Castenholz,1969;Stevens et al.,1985) and pH range of 4.8 to 9.8(Brock & Brock ,1970;Binder et al., 1972). Photosynthetic algae and cyanobacteria have been proposed for producing biofuels through a direct photoconversion process (Hongguang Lu., et al., 2011). In the present investigation Mastigocladus luminous has been isolated from hot water spring Tattapani at its native temperature 45 $^0$C, where as the maximum temperature of spring is $62^0$C. The temperatures and light intensity effect on the photosynthesis has been studied to generate the information for its use for biophotolysis purpose and to understanding of photosynthesis and distribution cyanbacteria in thermal spring

## Materials and Methods

Log phase M.laminosus cells (500μg protein ml-1) grown at $25^o$ C and $45^o$C were used to determine the photosynthetic $O_2$ evolution. Photo production of $O_2$ was measured by a calibrated Clark type-$O_2$ electrode enclosed in a 10ml air tight reaction vessel and connected to an oxygen analyzer (Universal Biochem, Model M 76T, India). The amount of $O_2$ evolved was expressed as μ mol $O_2$ mg-1 protein min-1.

**RuBP Case activity**

RuBP carboxylase was assayed by measuring RuBP dependent $^{14}CO_2$ fixation in to acid stable material. The buffer washed cyanobacterial pellets of temperature 25 $^{o}$C,35$^{o}$C and 45$^{o}$C were ground in glass powder with isolation buffer 5mM Tris, 1mM $Na_2$ EDTA; 1mM $MgC_{l2}$,, 20mM $NaHCO_3$ and 2mM GSH with a pH of 8.0). These were centrifuged and the supernatant were collected. 50µl of each of supernatants was transferred to a closed 1.5ml Ependorff vial and 25µl of preincubation mixture (50mM of NaHCO3 and 10mM $MgC_{l2}$ in 20mM Tris HCl buffer of pH 8.0) was added. The vials were closed and incubated at 30$^{o}$C. After 10 min the reaction was started by addition of 25ul of the assay mixture (2.19mM RUBP, 0.898mM $NaHCO^3$ with 1.27 uci of $^{14}$C radio activity and 10mM $MgC_{l2}$) and carried at 30$^{o}$C for 5min. After the assay period, the reaction was terminated by the addition of 400ul of 25% TCA solution and gently tapped to facilitate proper mixing. Assay without the addition of RuBP was also carried out and were treated as control.

100 µl of the assay solution was dried using a gentle hot air blow from on a Whatman No. 1 filter paper dishes of 2cm diameter and fumigated with HCl for an hour in fumigation chamber which ensured total removal of residual bicarbonate if present. The discs were then transferred to a counting vial and 5ml of scintillation cocktail (Ready value TM, Beckman, USA) was added. The vials were shaken at 150rpm for an hour and the radioactivity was counted using liquid scintillation counter (LS 1701, Beckman, USA). Window setting for $^{14}$C counting calibration were carried out in LS Counter prior to counting. The sample were counted for 5 min which ensured stability of radio emission with SCR (Sample Channel Ratio) as the qunich monitor and RCM (Random Coincidence Monitor) as the measure of Chemiluminescence and the counts were expressed as disintegrations per min (dpm).

Suitable blanks were also used while counting to determine background noise level to subtract from the counts of the sample. The enzyme activity was expressed as p mole of $CO^2$ fixed min-1 by calculation in comparison with radioactivity obtained with known molarity of $NaH^{14}CO_3$ standard. Enzyme units were expressed as p mole of $CO_2$ fixed min-1 and the specific activity in enzyme units per µg of protein.

## Results and Discussion

The results of time course studies of photosynthetic oxygen evolution in light in intact cells of 45$^{o}$C grown culture are shown in the Fig.1.

At all the temperatures, photosynthetic oxygen evolution in light linearly increased with time up to 5 minutes. Maximum oxygen evolution was observed at 45$^{o}$C, where the growth of organism was optimum. At suboptimal temperature (25$^{o}$C) and supraoptimal temperature (50$^{o}$C), the rate of photosynthetic $O_2$ evolution drastically declined. After successive culturing of the organism at sub-optimal temperature (25$^{o}$C) for 10 generations, the photosynthetic behaviour was observed again at different measurement temperatures (25$^{o}$C, 35 $^{o}$C and 45$^{o}$C). The rate of photosynthetic $O_2$ evolution was again highest at 45$^{o}$C in the cells pre-adapted to 25$^{o}$C for longer time. This suggested that shifting of the culture at

suboptimal temperature did not alter the photosynthetic behaviour of the organism as shown in Fig. 2.Comparative studies of quantum requirement for optimum $O_2$ evolution were made at measurement temperature of 45$^o$C using the pre adapted cells grown at low ( 25$^o$C) and high temperature 45$^o$C (Table 1). The rate of photosynthetic oxygen evolution up to 5 minutes was highest at light saturation 28wm$^{-2}$ in the organism grown previously at 25$^o$C while it decreased in the cells grown at 45$^o$C under similar light saturation conditions.

Photosynthesis is an important factor determining thermophilic growth of the blue-green algae. Maximum photosynthetic efficiency of thermophilic blue green algae taken from hot spring was observed at the high temperature of the natural habitat ( Brock,1967). The rate of photosynthetic $O_2$ evolution in thermophilic Synechococcus sp. was found high at the temperature range of 50$^o$C to 60$^o$C (Yamaoka et al.,1978). Photosynthetic membrane isolated from thermophilic *Mastigocladus laminosus* was active in electron transport through photosystem I and photosystem II as well as in photophosphorylation when cells were grown at 40$^o$C to 50$^o$C (Bohler & Binder, 1980). The higher quantum saturation for photosynthesis observed in cells grown at lower temperature is consistent with the earlier finding of Sheridan and Ulik (1976), where they found similar quantum saturation for thermophilic *Synechococcus livdus* cells grown at lower temperature. This finding suggests that both light and temperature have profound effect on photosynthesis of the cyanobacteria. At supraoptimal temperature, the high light intensity becomes lethal for photosynthesis. It is interesting to note that, the optimum rate of $O_2$ evolution was at 45$^o$C irrespective of the growth condition and light intensity (Fig2). The high light intensity (28wm$^{-2}$) which caused a reduction in the photosynthetic $O_2$ evolution in cells previously grown at 45$^o$C, enhanced the evolution in cells previously grown at 25$^o$C. In addition to this, the rate of $O_2$ evolution at measurement temperature of 50$^o$C in cells previously grown at 25$^o$C was almost compareable to the photosynthetic $O_2$ evolution rate determined at 45$^o$C in cells grown at 45$^o$C (Table 2).

Pearcy (1977) reported that changes in activity of RuBP Case with growth temperature resulted in proportional changes in $CO_2$ uptake. However in this study, $CO_2$ uptake was not measured. So, changes in the photosynthetic $O_2$ evolution at different light intensity and temperatures were not the indication of any alternation of the RuBP Case activity, but this the direct evidence of changes in light reaction of photosynthes is without involvement of carboxylating enzyme. In natural habitat *M. laminosus* and *S. lividus* have been reported to adapt at high temperature and low irradiance (Sperling,1975), which showed that these cyanobacteria optimize their photosynthesis relatively at low irradiance under thermal environment.

The RuBP Case activity in cell free extract of *M. laminosus* at various temperatures is shown in Fig. 3. The maximum activity was at 45$^o$C. The RuBP Case activity determined at suboptimal temperature of 25$^o$C and 35$^o$C exhibited lower activity at their respective growth temperatures in comparison to the activity at 45$^o$C grown cells as shown in

Fig.4. This suggested that $CO_2$ fixation catalyzed by the RuBP Carboxylase in thermal environment strictly requires a higher temperature comparable to habitat temperature for optimum growth and photosynthesis. The high RuBP Case activity and its stability up to 80$^oC$ in thermophilic *M. laminosus* have also been reported by Prasad (1988).reported in *Synechococcus leopoliensis* (Turpin et al., 1984) and *Anabaena chloroloeopsis* (Codd and Stewart,1976; Lanaras and Codd, 1981). The presence of RuBP carboxylase activity in the present strain of *M. laminosus* investigated showed that the organism possessed $C_3$ mode of carbon fixation and is quite active in thermal photosynthesis.

**Index of photosynthesis adaptation**

The adaptive photosynthetic potential of the organism was demonstrated by calculating the index of photosynthesis as shown in Table 1. Cyanobacterium *M. laminosus* was grown at 25, 35, 45 and 50$^oC$ for 10 generations. The rate of photosynthesis in terms of $O_2$ evolution was measured at each individual growth temperature and also at 50$^oC$. It was observed that organism grown at 25$^oC$ showed a higher Pmax (Maximum photosynthetic $O_2$ evolution) at 50$^oC$ and this Pmax decreased gradually in cells grown at temperature >25$^oC$. When rate of $O_2$ production at different growth temperatures is compared to the maximum rate achieved at 50$^oC$, some index of photosynthetic adaptation can be calculated. For example, the rate of $O_2$ production for the cells grown at 25$^oC$ was 0.8u mole $O_2$/mg protein/ minute at 25$^oC$. However, $O_2$ production by the same cells which measured at 50$^oC$ increased to 6.4µ mole $O_2$/mg protein/ minute.This is approximately 8 times greater than at 25$^oC$. The ratio represents a measure of potential increase in photosynthetic capacity achieved by the cells for adaptation to this high temperature. These potentials of photosynthetic adaptation for cels grown at 25$^oC$ -50$^oC$ are listed in Table 2.

of growth,the greater part of $CO_2$ assimilation in non-thermal cynobacteria has been reported to be catalysed by RuBP carboxylase and was detected in all cyanobacteria investigated(Smith, 1982). RuBP Case,the CO2 fixing enzyme of Calvin cycle is present in soluble and particulate forms in several blue-green algae. The particulate enzyme is principally present in the polyhydral bodies known as carboxysomes (Shively, 1974).

The result has been concluded in term of photo evolution of oxygen, index of photo adaptation and RuBP Carboxylase activity

**Photo evolution of oxygen**

Temperature response of photosynthesis of Matigocladus laminosus(isolated from hot water spring Tattapani, HP) was determined in terms of photo evolution of oxygen using cells grown at 45$^oC$. $O_2$ evolution from uniformly fragmented cells was tested at 25$^o$, 35$^o$, 45$^o$ and 50$^oC$. The maximum $O_2$ evolved (2, µ moles $0_2$/mg protein/5 min) at 45$^oC$, indicating the highest photosynthetic activity of this thermopile at test temperature of 45$^oC$ similar to the habitat temperature. The optimum temperature for photosynthetic oxygen evolution was found to be a genetic character which did not change in the cells of this thermopile strain pre adapted at suboptimum temperature (25$^oC$) for by repeated sub

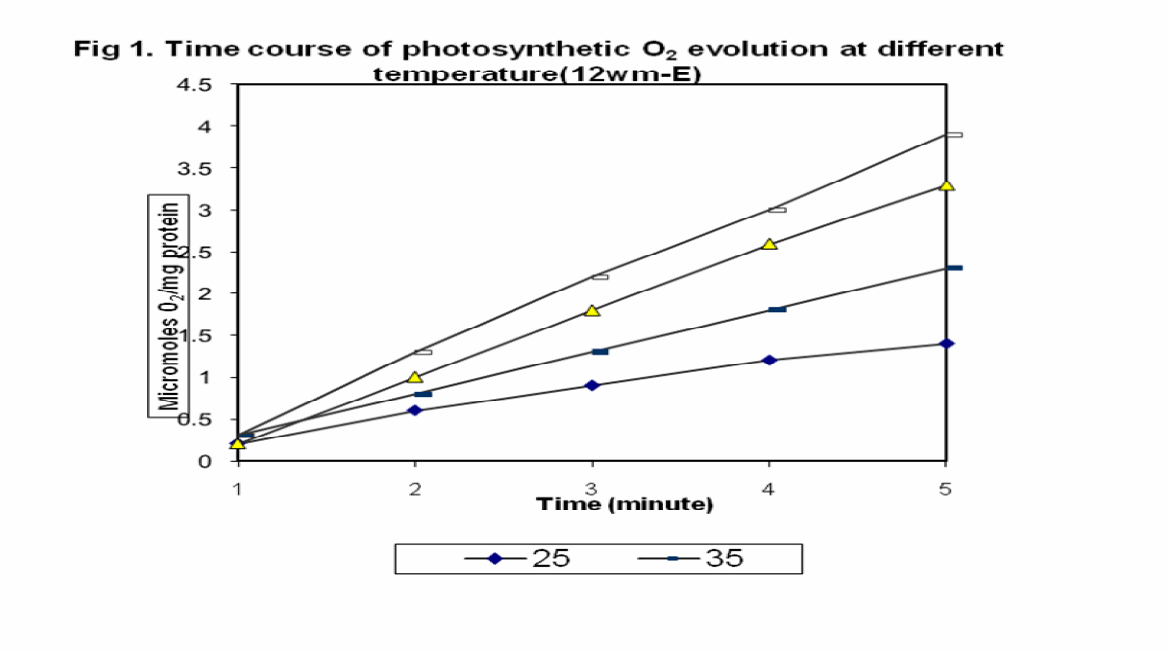



**Table: 1** Photosynthetic O2 evolution (measured at 45 $^0$C) at different light intensity ( E ) in pre adapted cells at temperature 25$^0$C and 45 $^0$C

| Pre adapted Growth Temperature ($^0$C) | Light intensity E (wm $^{-2}$ ) | $p^{max}$ (µ moll /mg protein at 45 ($^0$C ) |
|---|---|---|
| 25 | 2 | 0.6± 1.36 |
| | 4 | 1.0±1.00 |
| | 8 | 1.6 ± 0.55 |
| | 13 | 3.8±1.50 |
| | 28 | 4.2± 1.80 |
| 45 | 2 | 0.4± 0.72 |
| | 4 | 0.6±0.43 |
| | 8 | 1.2 ±0.25 |
| | 13 | 2.8±1.62 |
| | 28 | 2.1± 0.65 |

**Table 2** Index of photosynthetic adaptation

| **Growth Index Ratio Measured at growth $50^0C/O_2$-Evolution at growth temperature** | **Temperature(ºC) Measured at supra optimal Temperature ($^0C$)** | **P max (µ mole of $O_2$/mg protein/min) Maximum $O_2$ at temperature ($50^0C$)** | |
|---|---|---|---|
| 25 | 0.8 | 6.4 | 8.0 |
| 35 | 1.4 | 5.2 | 3.7 |
| 45 | 2.8 | 3.4 | 1.2 |
| 50 | 0.4 | 0.4 | 1.0 |

**Fig.2 Photosynthetic $O_2$ evolution at different temperatures in cells previously grown at 25ºC for 10 generations**

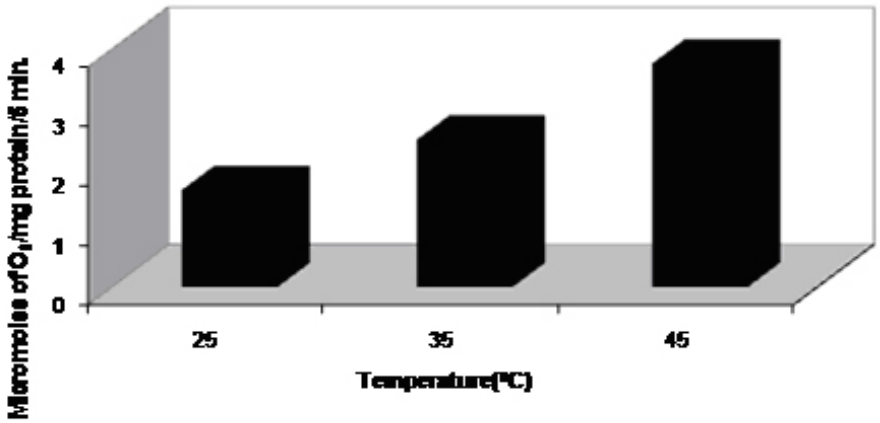



**Fig.3 RUBP carboxylase activity of Mastigocladus laminosus at different temperature grown at 45ºC**

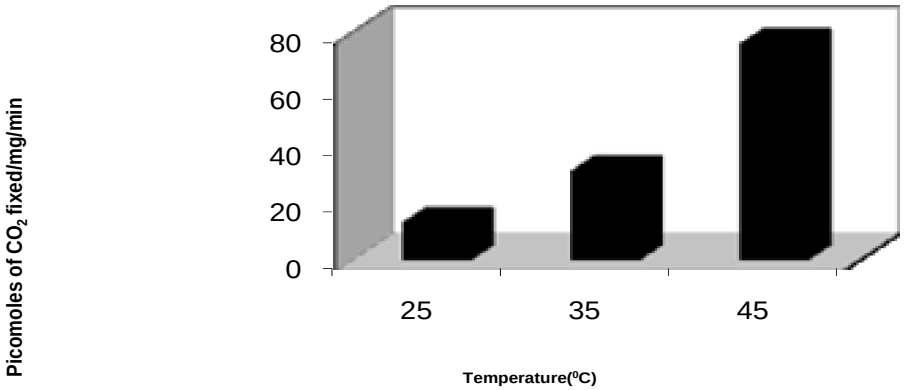
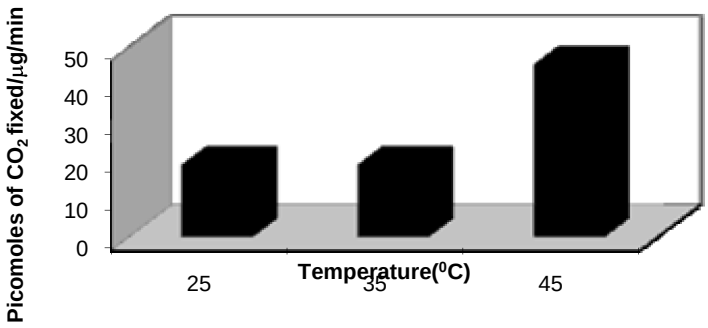


**Fig.4 RUBP carboxylase activity at different growth temperatures**




culturing at 25°C for a considerable period. Pre adapted cells at 25°C also showed maximum $O_2$ release in light at 45°C. However, 25°C grown cells showed relatively higher quantum saturation for $O_2$ evolution (28 W/m$^2$) compared to 45°C grown culture (13W/m$^2$). High temperature above 45°C and high irradiance both appeared to be photo inhibitory to photosynthesis. This also was reflected in its natural habitat, where the *M. laminosus* forms a thick mat adhered to the submerged rocks and is generally protected from direct sunlight and prefers to grow under diffused or low light intensity. This inability to tolerate high solar irradiance enables the organism to colonize more abundantly at various shady places of the hot springs.

## Index of photosynthetic adaptation

It was observed that photosynthetic rate suddenly became very high at test temperature of 45°C of cells growing previously at 25°C pre-adapted temperature). A high $O_2$ evolution rate of similar magnitude was not obtained at test temperature when cells grown at 45°C were used. The photosynthetic potential at test temperature of 45°C diminished gradually when higher temperature pre adapted cells were used (e.g. 35 to 45°C). In a common test temperature of 45°C when photo evolution of oxygen was compared using cells adapted both at 45°C and 25°C, it was observed that index of photosynthetic adaptation was higher (8.8) for 25°C compared to 45°C grown cells (1.2).

## RuBP Carboxylase activity

The presence of RUBP Carboxylase in cell free extract in the thermophilic strain of *M. laminosus* and its maximum activity detected at 45°C (approx. 72 pmoles of $CO_2$ fixed/ug chl-a/min) indicated the presence of $C_3$ mode of carbon fixation under thermal habitat. The activities measured at other two test temperatures of 25 and 35°C were approx. 12 and 28 pmoles $CO_2$ fixed/µg chl-a/min respectively. The activity was always maximum at 45°C irrespective of the growth temperature at which the cells were adapted/acclimatized for a considerable period. Thus it shows that RuBP Case in this organism has high affinity towards elevated temperature which is a prerequisite factor for thermal adaptation for photoautotrophs.

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