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# Effects of Various Physico-chemical Parameters on Growth, Morphology and Physiology of a Thermophilic Cyanobacterium *Fischerella thermalis* (Schwabe) Gomont

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**Abstract:** The growth as well as morphological and physiological features of thermophilic cyanobacteria vary greatly depending upon the physico-chemical factors prevailing in the water of hot springs. Effects of some physico-chemical parameters of water like-temperature, pH, nitrate and phosphate contents were observed on a thermophilic cyanobacterium *Fischerella thermalis* isolated from a hot water spring located at Panifala, Burdwan, West Bengal. Catalase activity under varying temperatures and light intensities was also studied in *in vitro* condition. Filaments incubated at 45°-55°C temperature, acidic (5.0-7.0) and highly alkaline (10.0) pH, high concentration (1.0-2.0 g/L) of $NaNO_3$ and low (0-0.02 g/L) as well as high concentration (0.1 g/L) of $K_2HPO_4$ showed various morphological abnormalities like shrinkage of cells, degradation of cellular contents, twisted and broken trichomes, discolouration of filaments, empty filaments. Such variations in physical and chemical factors also resulted in variations in protein, carbohydrate, chlorphyll-a and carotenoid contents. Heterocyst frequency and branching frequency was highest at 37°C temperature, 8.0-9.0 pH and 0.06 g/L of $K_2HPO_4$ concentration. At increasingly high concentration of $NaNO_3$ branching frequency increased but heterocyst frequency decreased. 37°C temperature, pH 8.0, 1.0 g/L $NaNO_3$ and 0.06 g/L of $K_2HPO_4$ concentration were found to be ideal for growth in *in vitro* condition. High light intensity (75 photons $m^{-2}$ $s^{-1}$) and low temperature (25°C) were observed to be responsible for increased catalase activity. This investigation will help to optimize the culture medium for *in vitro* production of biologically significant organisms like filamentous thermophilic cyanobacteria and throw light on experimental taxonomy.

**Key words:** Thermophilic cyanobacteria, physico-chemical parameter, Panifala, temperature, pH, nitrate, phosphate, catalase activity.

## Introduction

Geothermal springs are one of the extreme natural habitats where cyanobacteria exhibit high abundance forming multilayered thick colourful mats submerged in the spring water. One of the most significant features of the hot springs is that the temperature and water chemistry at the source remains constant [8]. This shows that any thermophilic cyanobacterium growing in thermal springs favours an optimal growth temperature at any season. However, preliminary observation indicates that there are possibilities of variations in different growth factors along the course of flow. For example, due to change in temperature along the flow of water there is change in pigment profile. Similarly, other physico-chemical parameter may also exert positive or negative influence upon species distribution throughout the year [8]. Differences in cyanobacterial distribution and diversity observed in the run-off water among springs or in a spring during different seasons are due to differences in chemical composition and temperature [44]. The pH is a very important parameter regarding the distribution of cyanobacteria in alka-

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line and neutral springs upto 75°C [6], but only to 55°C in acidic springs [12]. The nitrogen fixing ability of cyanoabcteria is reduced in conditions characterized by high nitrate concentration [14]. Heterocyst frequency of these organisms is also altered in nitrate rich environment [1]. Inorganic phosphate analysis of thermal water often shows much higher values than those found in surface freshwater [8,39] indicating its necessity for the growth and nitrogen fixation of thermophilic cyanoabcteria [32].

The enzymatic component of the antioxidant system is one of the defensive mechanisms providing protection against excessive light absorption in cyanobacteria. Cyanobacteria are among the first organisms to elaborate mechanisms for the detoxification of partially reduced oxygen species including $H_2O_2$ [4].

It is important to note that the thermophilic cyanobacteria have emerged as a promising bioresource for the production of several bioactive compounds. Efforts have been made to screen these microorganisms for the production of antibacterial compounds. *Mastigocladus laminosus* was found to produce antibacterial component [5]. Thermophilic cyanobacteria also have the potential to remove nutrients from thermal effluents [46]. Thermophilic *Synechococcus* sp. is a potential producer of poly-α-hydroxybutyrate, which is the basis of biologically degradable plastics [30].

Considering the important contributions of thermophilic cyanobacteria in hydrothermal ecosystems and its importance in producing thermostable bioactive compounds, a systematic study of the various parameters on growth and different growth parameters has been undertaken on a particular cyanobacterium *Fischerella thermalis*. This cyanobacterium plays an important role in maintaining the nitrogen status in hot springs [40]. As thermophilic cyanobacteria grow in one of the harshest climatic conditions of the world, their morphometric features may alter depending upon the nature and physico-chemical environment of the prevailing habitat. This study included a detailed morphometric analysis and response of the organism with regard to the amount of Chlorophylla, carotenoids, protein and carbohydrate production under different physical as well as chemical conditions. The effect of the varying ranges and concentration of these factors on the growth of thermophilic cyanobacteria will be elucidated by this study. This will also enable us to optimize the growth medium for production of thermophilic cyanobacteria under culture condition.

## Materials and methods

### *Sample collection and culturing*

Cyanobacterial mat samples used for this study were collected from a solitary geothermal spring at Panifala (23°45'33"N, 86°58'54"E) in the district of Burdwan, West Bengal, India from a concrete slab covered with ceramic tiles and from the run-off water. Collected mats were stored in zipper pouches and sterilized screw capped bottles. Mats were repeatedly washed with distilled water and put into agarised (1.2 % w/v) BG-11 (-N) medium in Petri-plates, incubated at 37±2°C under 30-35 µmol photons $m^{-2}$ $s^{-1}$ light (12 h:12 h Light and dark cycle). Unicyanobacterial cultures of *F. thermalis* were established by repeated subculturing method.

### Experiments with variation of parameters

Along with varying the temperature and pH, the effect of phosphate and nitrate on cyanobacterial growth was studied by varying the concentrations of $K_2HPO_4$ and $NaNO_3$ respectively in BG 11 medium. Experimental organisms were put into culture media at various temperatures ranging from 25-55°C, pH ranging from 5 to 10, different concentrations of $K_2HPO_4$ (0 to 2.0 mg $L^{-1}$) and $NaNO_3$ (0 to 0.1 mg $L^{-1}$).

### Modification of culture medium

To study the effect of varying ranges of temperature, pH and various concentrations of nitrate and phosphate following ranges or concentrations were used wherever applicable and the culture medium was modified accordingly-

Temperature: 25°C, 37°C, 45°C and 55°C. 37°C was set as control.

pH: 5, 6, 7, 8, 9 and 10. pH 8 was considered as control.

Nitrate ($NaNO_3$): 0 g/l, 0.5 g/l, 1 g/l, 1.5 g/l and 2 g/l. BG-11 medium without $NaNO_3$ was considered as control.

Phosphate ($K_2HPO_4$): 0 g/l, 0.02 g/l, 0.04 g/l, 0.06 g/l, 0.08 g/l and 0.1 g/l. 0.04 g/l was set as control.

**Study of morphological features**

The organism was morphologically studied and identified following Rippka *et al*.[34] and Anagnostidis & Komarek [2]. Microphotographs of morphological abnormalities because of various treatments were taken with Leica trinocular DM 2500 research microscope. Study of generation time was done following Guillard [17]. Major morphological changes like shape and dimension of cells, filaments, sheath, heterocysts, breakage of filaments, etc. were noted along with changes of temperature, pH, nitrate and phosphate concentration. Frequency of branching, heterocyst, filament breakage, formation of hormogonia and akinete and any other morphological abnormality were studied following Kaushik [25]. Heterocyst frequency (H %) was calculated by number of heterocyst present per hundred vegetative cells.

**Study of physiological characteristics**

To study the effects of temperature, pH and different concentrations of $NaNO_3$, $K_2HPO_4$ following parameters were considered to be most relevant in case of thermophilic cyanobacteria - Chlorophylla content, Carotenoids content, protein content, carbohydrate content, growth and morphological parameters like frequency of heterocyst, true branching and filament breakage. Chlorophylla (Chl *a*) was determined following the methodology of Mackinney [29]. Total carotenoids concentration was measured spectrophotometrically at 450 nm and the carotenoids: chlorophylla ratio was determined. Protein estimation was done following the method of Lowry *et al*. [28]. Carbohydrate assay was done following Herbert et al. [18]. Growth was turbidometrically measured at 750 nm.

**Statistical analysis**

Two-tailed t-Test was performed to test the significance of the differences between the mean values for carotenoids, chlorophyll-a and their ratio, proteins, carbohydrates and growth on various treatments. Single-factor ANOVA was performed to assess the variance of these parameters.

**Study of catalase activity**

Unicyanobacterial culture of *F. thermalis* was incubated at 3 different temperatures like 25, 37 and 45°C. To assess the catalase enzyme activity under varying light intensities, organisms were incubated at 30 and 75 photons $m^{-2}$ $s^{-1}$ light. Activity of catalase enzyme was assayed in *F. thermalis* following Kar and Mishra [23] with some modification by Kar and Choudhuri [24]. Enzyme activity was measured following the formula of [14].

**Acetylene reduction assay**

Nitrogenase activity is assayed by acetylene reductiob assay (ARA) method using a gas chromatograph (HP) fitted with Porapack N (80 - 100 mesh) column following the method of Turner and Gibson [43].

**Results**

The generation time of cultured strain of *Fischerella thermalis* was found to be 18 days. Effects of temperature, pH and different concentrations of sodium nitrate and di-potassium hydrogen phosphate on various parameters of *F. thermalis* are described below:

**Effect of temperature**

Branching frequency was highest at control temperature (Table 1). Heterocyst frequency decreased in both lower (25°C) and higher (45-55°C) temperatures with respect to control (37°C). No morphological abnormality was found in 25°C and 37°C. But various abnormalities such as breakage of filaments, discolouration of mass, coming out of cellular matrix outside the cell, empty cells and filaments were found at higher temperature regime.

Study of physiological features revealed that growth (in terms of Chl-a and turbidity) increased upto 37°C (control) and then markedly decreased with increasing temperature (Fig. 1). The increase in growth from 25°C to 37°C (control) is significant at á < 0.01. Growth was ceased at 55°C. Carotenoids content increased upto 45°C following increasing temperature. Protein content exhibited increasing tendency upto 37°C, but showed a relatively slight increase at 55°C after a sharp decrease at 45°C (Fig. 2). Carbohydrate content was highest at 25°C and gradually decreased with increas-

**Table 1. Effect of temperature on morphology of *F. thermalis***

| Temperature (°C) | Branching Frequency (%) | Heterocyst Frequency (%) | Filament breakage | Morphological abnormalities |
|---|---|---|---|---|
| 25 | 18.3 | 3.12 | No | No |
| 37 (control) | 31.6 | 6.87 | No | No |
| 45 | 8.6 | 2.37 | Rare | Discoloured mass, empty cells, granular cellular matrix outside the trichome |
| 55 | 6.2 | 1.8 | Yes | empty cells, granular cellular matrix outside the trichome |

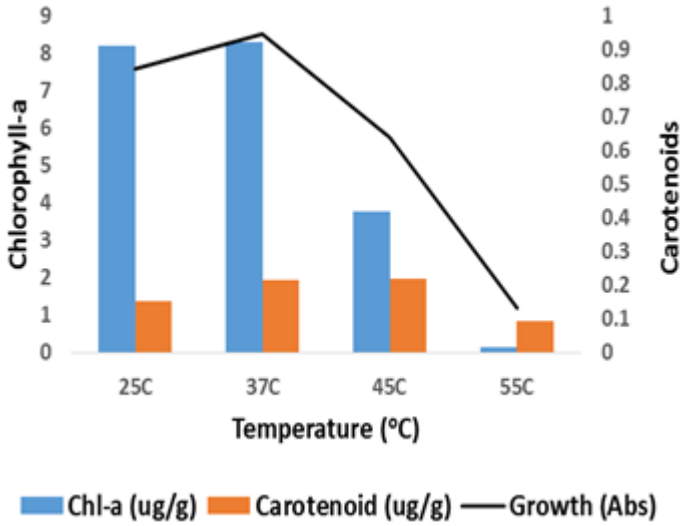


**Fig. 1.** Effect of temperature on growth, Chl-a and carotenoid contents of *F. thermalis*

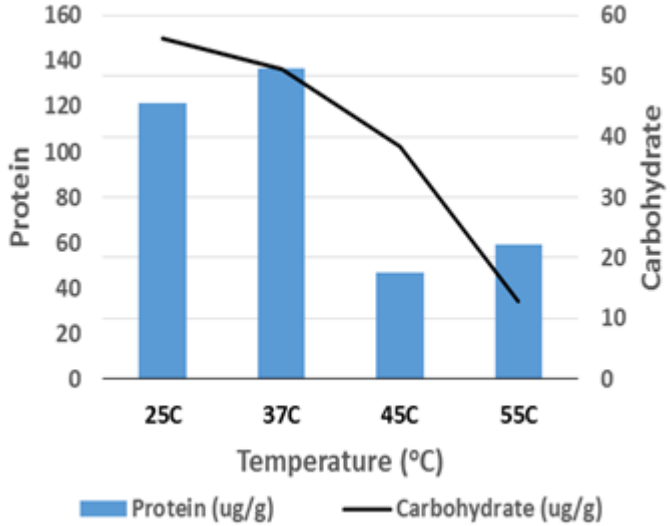


**Fig. 2.** Effect of temperature on protein and carbohydrate contents of *F. thermalis*

ing temperature (Fig. 2). Result of single-factor (temperature range: 25-55°C) ANOVA indicated that the impact on protein, carbohydrate, Chl-*a*, carotenoids and growth of *F. thermalis* is significantly ($p < 0$) different.

**Effect of pH**

Branching frequency gradually increased upto pH 8.0 (control) as it was recorded highest (34.48 %) at this pH (Table 2). It slightly decreased at pH 9 (28.5 %) and 10 (23.53 %). Heterocyst frequency was highest (6.17 %) at pH 9.0 and gradually decreased up to 1.76 % at pH 5.0 (Table 2). Breakage of filaments occurred at lower pH values below 8.0. No morphological abnormalities were found at pH 8.0 and 9.0 (Plate 1, fig. A). But various abnormalities such as - shrinkage of cells, absence of cellular matrix in cells, empty cells and filaments, twisted and broken trichome and thickening of trichomes at some places were found at lower pH at 5.0 to 7.0 as well as higher pH at 10.0 (Plate 1, figs. B-D).

Study of physiological features in varying pH (Figs. 3 and 4) revealed that growth (in terms of Chl-a content) was low at lower pH and reached the highest at pH 8.0. Protein content also reached the highest at pH 9.0 following a gradual increase from acidic range. Carbohydrate and carotenoids contents were also found to express gradual increase from acidic range towards highest amount at alkaline pH of 8. Most of the studied parameters like, carbohydrates, Chl-a and carotenoids exhibited a slight decline in the contents at pH 9 and 10. Protein content also slightly decreased at pH 10. The increase in growth from pH 7 to 8 (control) was significant at $\alpha < 0.1$ and the decrease in amount of carbohydrate and protein from control to pH 7 and 9 was significant at $\alpha < 0.1$. The result of single-factor (the range of pH 5.0-10.0) ANOVA indicates that the impact on protein, carbohydrate, Chl-a, carotenoids and growth of treated strain of *F. thermalis* is significantly ($p < 0$) different here also.

**Effect of nitrate ($NaNO_3$)**

Branching frequency gradually increased from control (0 g/L $NaNO_3$) to a highest of 51.2 % at 2 g/L $NaNO_3$ (Table 3). Heterocyst frequency gradually decreased from control (14.67 %) to 2 g/L $NaNO_3$ (1.17 %) with increasing concentration of

**Table 2. Effect of pH on morphology of *F. thermalis***

| pH | Branching Frequency (%) | Heterocyst Frequency (%) | Filament breakage | Morphological abnormalities |
|---|---|---|---|---|
| 5 | 7.5 | 1.76 | Yes | Shrinkage of cells, often absence of cellular matrix; empty filaments |
| 6 | 14.5 | 2.70 | Yes | Shrinkage of cells, degeneration of cellular contents; sometimes empty filaments |
| 7 | 16.6 | 2.98 | Yes | Twisted trichome, broken trichome; discoloured filaments; sometimes empty cells |
| 8 (control) | 34.48 | 4.46 | No | None |
| 9 | 28.5 | 6.17 | No | None |
| 10 | 23.53 | 3.20 | No | Degeneration of cellular contents; filaments thickened at places, seldom empty filaments |

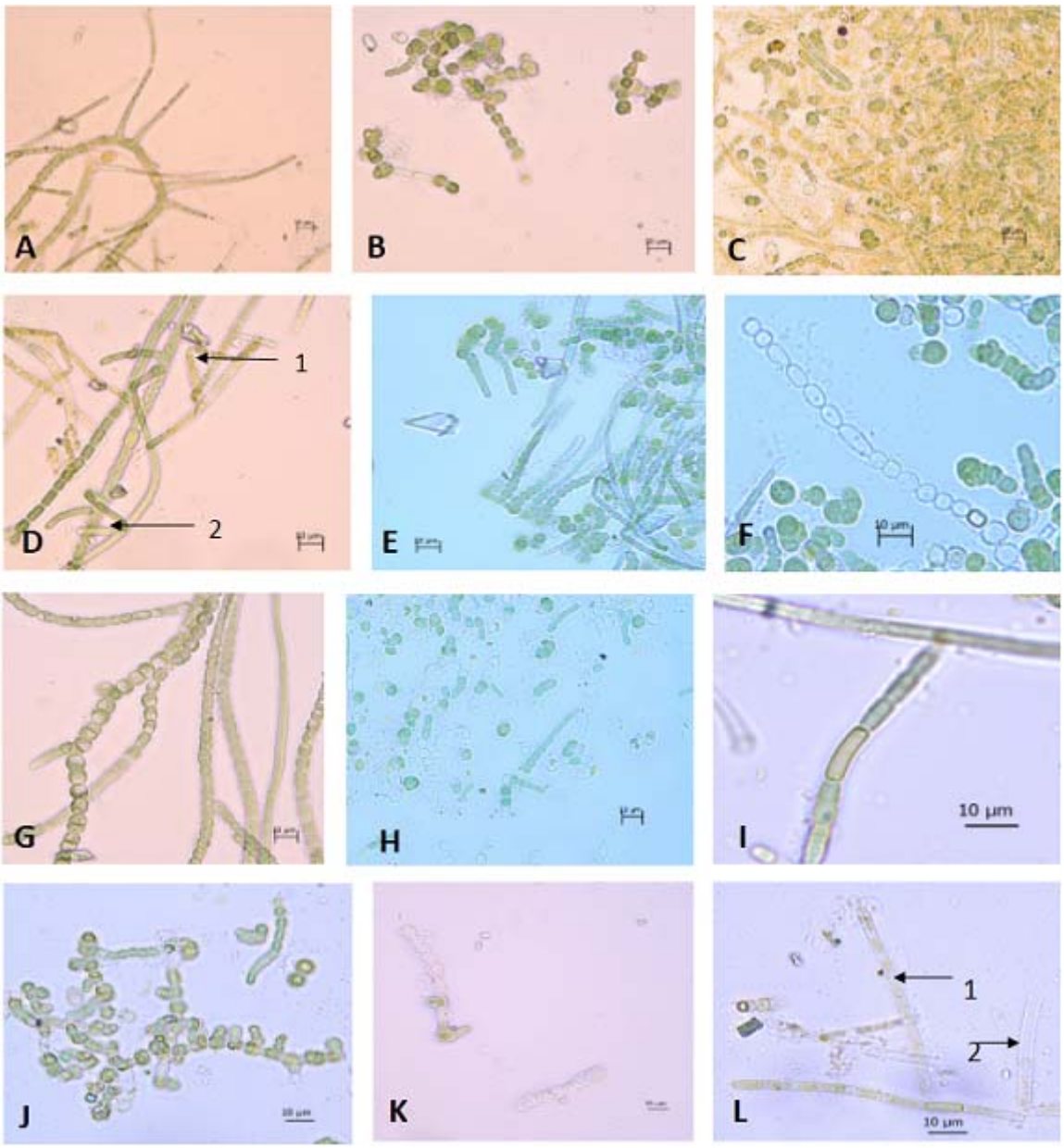


**Plate 1: Fig. A.** Normal trichome of *F. thermalis* at pH 8.0; **fig. B**. Empty cell at pH 5.0; **fig. C.** Discoloured and empty filaments at pH 6.0; **fig. D.** 1. coiled filament; 2. Distorted tip of the filament; **fig. E.** Filaments showing high frequency of branching at 1.0 g/L nitrate; **fig. F.** Empty filament at 0.5 g/L nitrate; **fig. G.** Degraded cellular materials at 2.0 g/L nitrate; **fig. H.** Filaments at 45°C; **fig. I.** Large heterocyst at 0.06 g/L phosphate; **fig. J.** High branching frequency at 0.8 g/L phosphate; **fig. K.** Empty trichome at 0.1 g/L phosphate; **Fig. L. 1**. Discoloured and 2. hollow filament at 0.1 g/L phosphate concentration (Scale bars: 10 µ).

$NaNO_3$ (Table 3). No morphological abnormality was found in 0 g/L $NaNO_3$. Empty filaments were very rarely observed in 0.5 g/L $NaNO_3$. But degradation of cellular contents and empty filaments were frequently found at the concentration 1 to 2 g/L $NaNO_3$ (Plate 1, figs. E-G).

Study of physiological characteristics in varying concentrations of $NaNO_3$ (Figs. 5 and 6) revealed that growth was highest at 1.0 g/L $NaNO_3$. Protein content showed increased amount (278.4

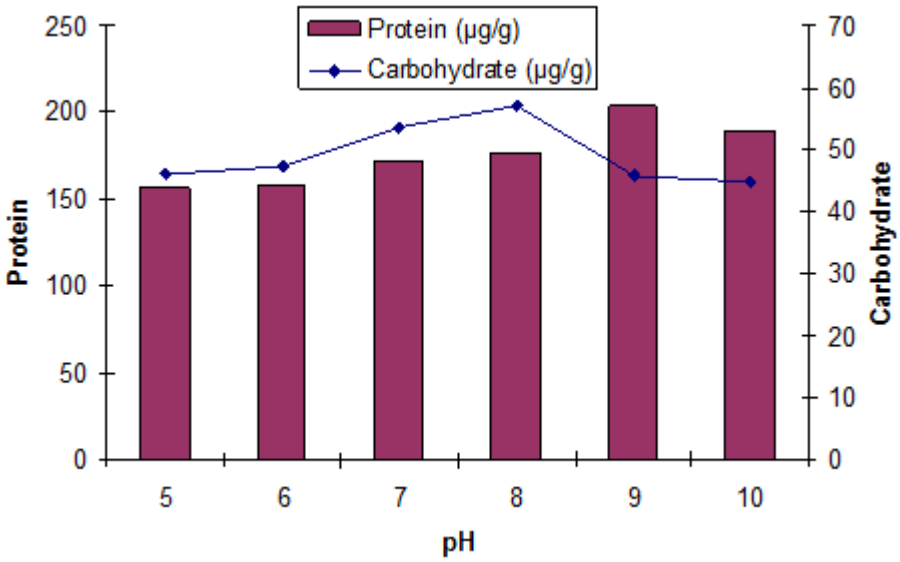


**Fig. 3.** Effect of pH on protein and carbohydrate contents of *F. thermalis*

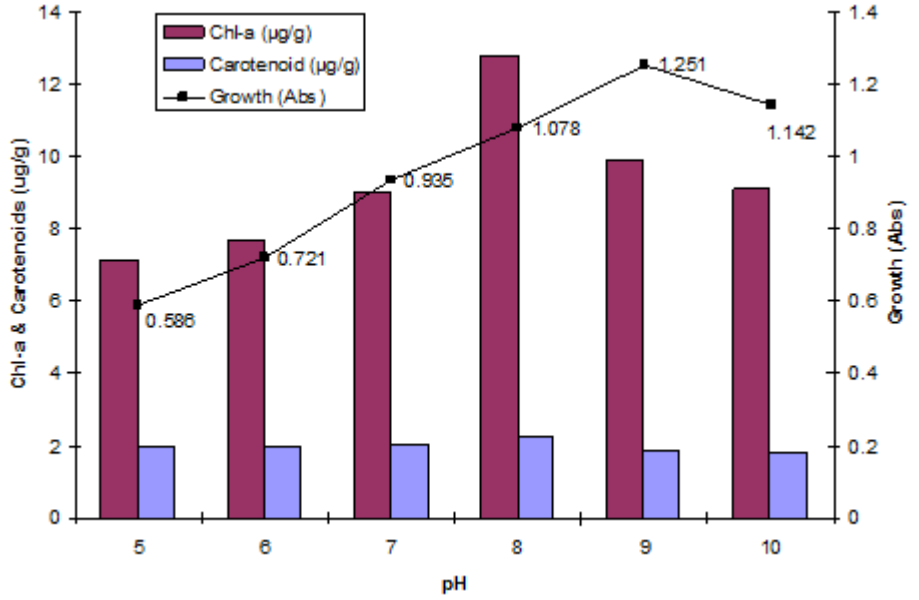


**Fig. 4.** Effect of pH on growth, Chl-a and carotenoid contents of *F. thermalis*

µg/g) at 0.5 g/L $NaNO_3$ concentration and gradually decreased with increasing $NaNO_3$ concentration. Higher expression of carbohydrate, Chl-a and carotenoids was observed at 1.0 g/L $NaNO_3$ which gradually decreased with decreasing as well as increasing concentrations of $NaNO_3$. Gradually increased production of Chl-a at 1.0 g/L $NaNO_3$ was significant at $\alpha < 0.05$. Increase in amount of protein from 0 to 0.5 g/L $NaNO_3$ was significant at $\alpha < 0.01$. Increase in amount of carbohydrate at 1.0 g/L $NaNO_3$ was significant at $\alpha < 0.01$. The result of single-factor (different concentrations of $NaNO_3$) ANOVA indicated that the impact on protein, carbohydrate, Chl-a, carotenoids and growth of treated strain of *F. thermalis* is significantly ($p < 0$) different.

**Effect of phosphate ($K_2HPO_4$)**

Morphological study revealed that branching frequency was high (22.1 %) at 0.06 g/L $K_2HPO_4$ which gradually decreased with increasing concentration of $K_2HPO_4$ (Table 4). Heterocyst fre-

**Table 3. Effect of $NaNO_3$ on morphology of *F. thermalis***

| $NaNO_3$ concentration (g/L) | Branching Frequency (%) | Heterocyst Frequency (%) | Filament breakage | Morphological abnormalities |
|---|---|---|---|---|
| 0 (control) | 5.35 | 14.67 | No | - |
| 0.5 | 8.23 | 8.48 | No | Very rarely empty filaments |
| 1.0 | 17.10 | 3.50 | No | Partial degradation of cellular contents, rarely empty filaments |
| 1.5 | 36.39 | 1.17 | Rare | Partial degradation of cellular contents, empty filaments |
| 2.0 | 51.20 | 0.93 | Yes | Degradation of cellular contents, frequent empty filaments |

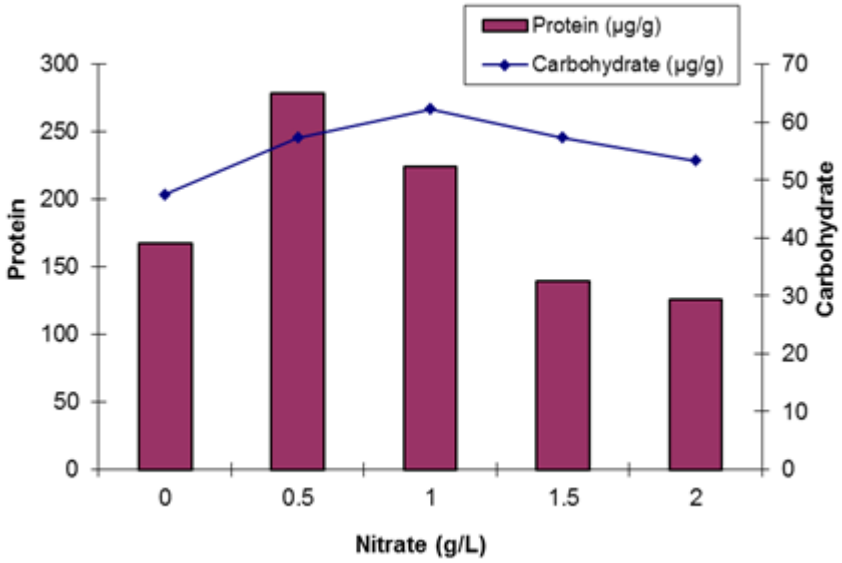


**Fig. 5.** Effect of $NaNO_3$ on protein and carbohydrate contents of *F. thermalis*

quency also was high (11.2 %) at this very concentration of $K_2HPO_4$ that gradually decreased with increasing and decreasing concentration of $K_2HPO_4$. No morphological abnormality was observed at 0.04 g/L $K_2HPO_4$. But various deformities such as-breakage of secondary lateral filaments, empty cells, enlarged heterocysts, contents coming out of cells, discolouration and degradation of filaments, etc. were observed at lower concentrations (0 and 0.02 g/L) than control as well as higher concentrations than control (0.06, 0.08 and 0.01 g/L) (Plate 1, figs. I-L).

Study of physiological characters (Figure 7 and 8) revealed that growth was high at control concentration (0.04 g/L) which gradually decreased with decreasing and increasing concentrations of $K_2HPO_4$. The increase of growth from 0.02 g/L to 0.04 g/L (control) was significant at $\alpha < 0.05$. Higher amount of protein (46.4 µg/g), carbohydrate (79.2 µg/g), Chl-a (9.19 µg/g) and carotenoids (1.13 µg/g) was observed at 0.04 g/L $K_2HPO_4$. These parameters were low at phosphate starved condition (0 and 0.02 g/L $K_2HPO_4$) and increased at 0.04 g/L $K_2HPO_4$. The increase of

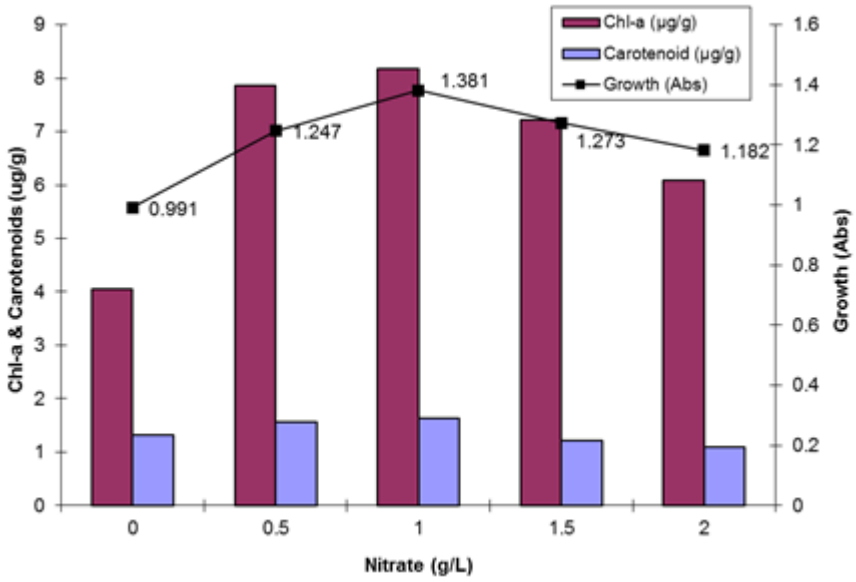


**Fig. 6.** Effect of $NaNO_3$ on growth, chl-a and carotenoid contents of *F. thermalis*

**Table 4. Effect of $K_2HPO_4$ on morphology of *F. thermalis***

| $K_2HPO_4$ concentration (g/L) | Branching Frequency (%) | Heterocyst Frequency (%) | Filament breakage | Morphological abnormalities |
|---|---|---|---|---|
| 0 | 4.68 | 1.10 | Yes | Broken secondary laterals, contents coming out of cells |
| 0.02 | 14.83 | 6.40 | No | Rarely contents coming out of cells |
| 0.04(control) | 18.72 | 9.36 | No | - |
| 0.06 | 22.10 | 11.20 | No | Enlarged heterocysts |
| 0.08 | 8.20 | 7.41 | Yes | Partially discoloured and degraded filaments, brownish trichome |
| 0.1 | 6.14 | 4.79 | Yes | Empty trichome, discoloured and degraded filaments, colour becomes brownish |

carbohydrate and protein from 0.02 g/L to 0.04 g/L was significant at $\alpha < 0.01$. The amount of protein, carbohydrate, Chl-a and carotenoids again decreased at higher concentrations of $K_2HPO_4$ (0/06, 0.08 and 0.1 g/L) (Figs. 7 and 8). The result of single-factor (different concentrations of $K_2HPO_4$) ANOVA indicated that the impact on protein, carbohydrate, Chl-a, carotenoids and growth of treated strain of *F. thermalis* is significantly ($p < 0$) different.

**Catalase activity**

The experimental organism, *F. thermalis* exhibited decreasing tendency in catalase activity with the increase of temperature. Highest activity of catalase enzyme was observed at 25°C (Fig. 9). High light intensity (75 photons $m^{-2}$ $s^{-1}$) was found to be more effective to produce elevated level of

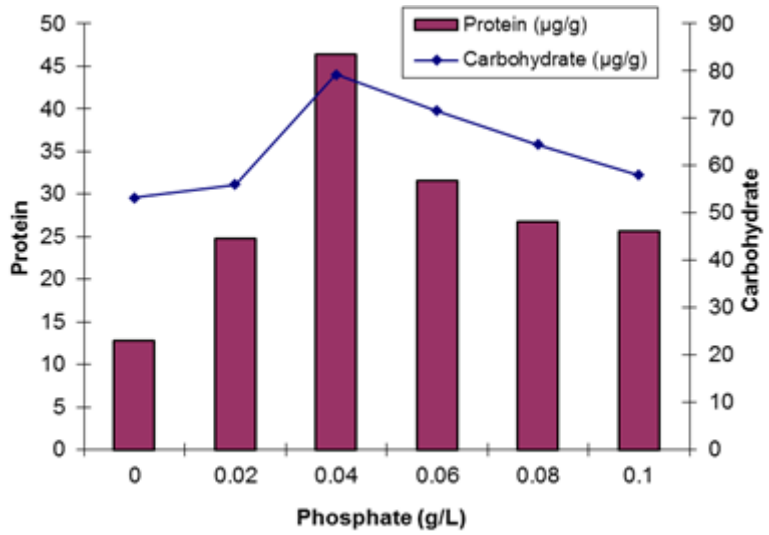


**Fig. 7.** Effect of $K_2HPO_4$ on protein and carbohydrate contents of *F. thermalis*

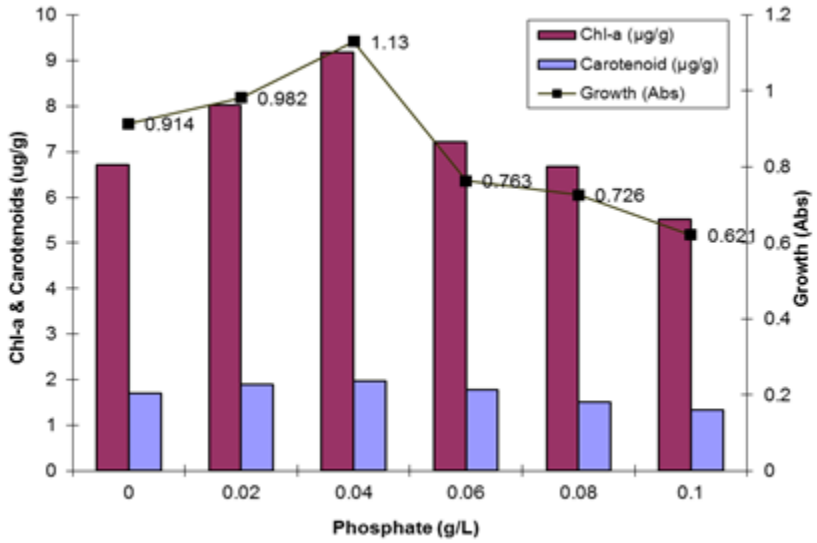


**Fig. 8.** Effect of $K_2HPO_4$ on growth, chl-a and carotenoid contents of *F. thermalis.*

catalase activity rather than normal light intensity (30 photons $m^{-2}$ $s^{-1}$) that is ideal for *in vitro* culturing of cyanobacteria (Fig. 10).

## Discussion

Thermophilic cyanobacteria are one of the most important components of the microbial flora of hot springs [6,7,8,12,39,45,46]. **T**homas and Gonzalves [42], Vasistha [43], Jana [19], Jha [20], Jha and Kumar [21], Adhikary [1], Debnath *et al.* [11] and Roy *et al.* [35,36] have systematically studied the cyanobacterial flora of hot springs from various parts of India.

The results of the present study indicated that variations in temperature and parameters like pH, concentration of nitrate, and phosphate in growth medium have direct effects on the growth and metabolism of thermophilic cyanobacteria (Tables 1-4 and Figs. 1-8). This has been reflected in morphological abnormalities, variation in expression of taxonomically important features and protein, carbohydrate, Chl-*a* and carotenoids contents.

Some stigonematalean species of cyanobacteria have been reported to grow naturally either at the sources having a temperature as high as 60°C or at down streams of thermal springs [19,46] but they

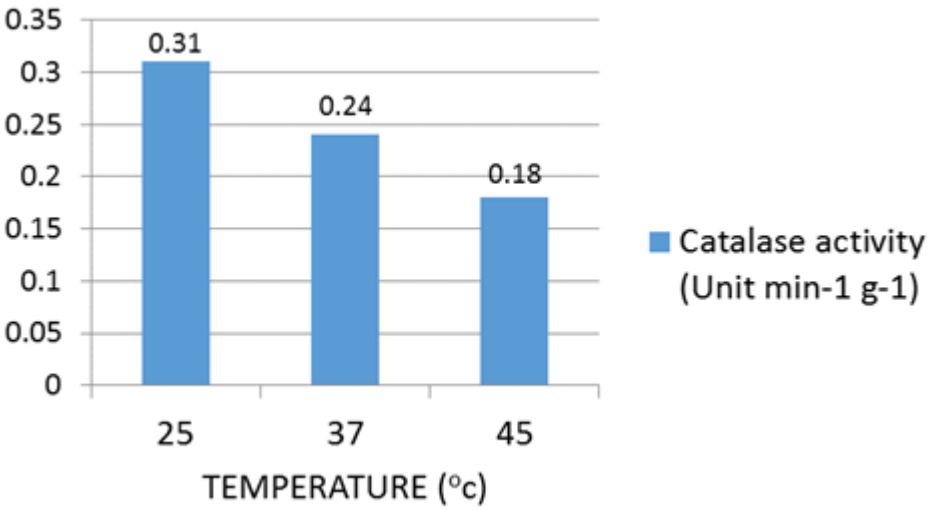


**Fig. 9.** Effect of varying temperatures on Catalase activity of *F. thermalis*

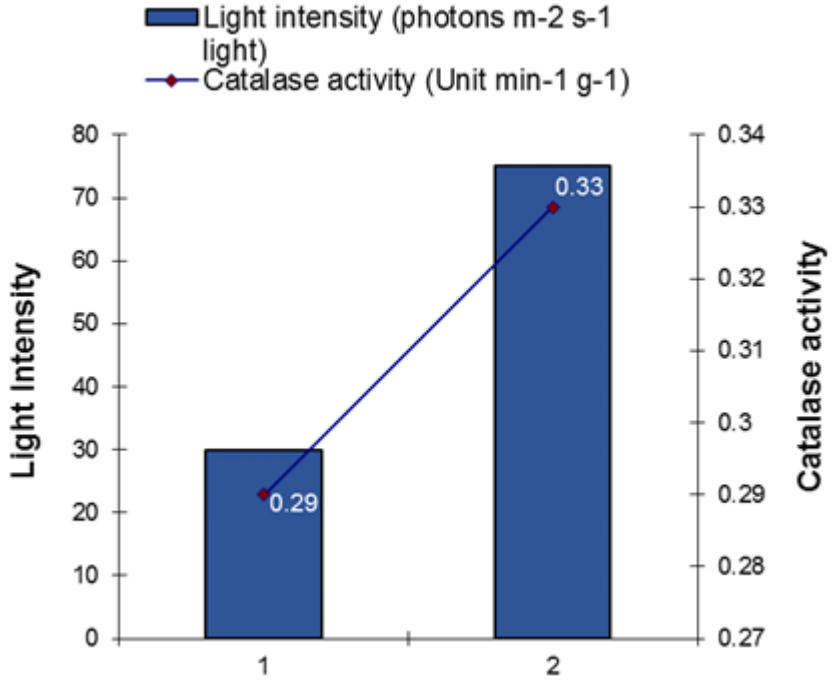


**Fig. 10.** Effect of varying light intensities on Catalase activity of *F. thermalis*

generally lose the ability to tolerate high temperature under culture condition. These strains could have undergone major alterations in features in response to culture medium and culture condition. This shift in temperature tolerance is related to membrane function which is responsible for providing thermostability [21]. In the present study, *F. thermalis* grew profusely at 37°C in the BG-11 (-N) medium. Growth ceased at 55°C which is optimal temperature for natural thermophilic populations. 37°C, though being the sub-optimal temperature for thermophilic cyanobacterial growth, has been established as ideal temperature for culture purposes of this species. This supports the works of Castenholz [8,9] regarding the *in vitro* cultivation and maintenance of high temperature race of *Mastigocladus laminosus* at over 60°C followed by cloning with agar shake or streak methods at 45°C.

Most cyanobacteria have optimal growth at pH

between 7.5 and 10.0 [14]. Bano and Siddiqui [3] measured Chl-*a* content and total proteins of five marine cyanobacterial species and concluded that those organisms preferred near-neutral to alkaline pH. They measured growth in terms of Chl-*a* and protein contents under different pH. *Spirulina major,* a filamentous cyanobacteria exhibited highest growth in terms of Chl-*a* content at pH 6.5, whereas highest growth was obtained at pH 8.0 in terms of total protein content. In present study, highest turbidometric growth rate was recorded at pH 9 and highest amount of Chl-a was recorded at pH 8. This corroborates the findings of earlier workers [3,20]. Low pH may block cell division and septation by acting externally on cell membrane or cell wall [22]. A defect in solute transport at low pH may be the immediate cause of growth and viability loss [22]. In the present study, shrinkage of cells, degradation of cellular matrix occurred at pH 5 and 6. But no such abnormalities occurred at pH 8 and 9 (Table 2). Lesser growth rate was also observed at lower pH (Fig. 4). These findings support the earlier observations regarding the effect of low pH.

Ammonium-$N_2$ often leads to poorer growth and may even cause cell lysis whereas nitrate supplied in the medium supports growth to some extent at comparable levels [3]. Castenholz [8] reported that spring sources deficient in combined nitrogen allow nitrogen fixing thermophilic cyanobacteria *Mastigocladus* to dominate, since no other thermophiles of higher temperature range are capable of nitrogen fixation. Abundance of nitrogen-fixing cyanobacterial species is negatively correlated with inorganic $N_2$ contents, indicating the ability of these species to colonize at low inorganic $N_2$ concentration [10,13]. Ernst *et al*. [13] documented that increasing nitrate concentrations delayed growth of two phycoerythrin-rich strains of *Synechococcus* sp., even at low initial phosphate level. Here also it has been found that growth (in terms of Chl-a and turbidity) in the experimental organism has decreased at higher concentrations (1.5-2.0 g/L) of $NaNO_3$ (Fig. 6). In this experimental material we have found that the ARA value of this organism is 0.312 n mol $C_2H_4$/µg of Chl-*a*/h. This result shows similarity with earlier reports.

Kuffner and Paul [26] reported enhanced growth of *Lyngbya majuscula* with phosphate enrichment in the nutrient medium. Shikha *et al.* [37] observed that Chl-a content of wild type and mutant strains of *Anabaena doliolum* increased in presence of supplemented inorganic phosphate and declined under phosphate starved condition. Ernst *et al*. [13] showed that, low concentration of $K_2HPO_4$ (0.03 g/L) inhibited growth of *Synechococcus* sp. Yandige**ri** *et al.* [48] cultured *Westiellopsis prolifica* and *Aanbaena variabilis* in phosphate-starved condition for 15 days and observed that the total Chl *a* content, total soluble protein content and total carbohydrate were lower at phosphate starved condition at 14$^{th}$ day than the organisms grown in normal BG-11 medium with $K_2HPO_4$. In present study also, 18 day's old culture of *F. thermalis* showed lower amount of the Chl-*a*, carotenoids, carbohydrate and protein contents as well as lesser heterocyst frequency at phosphate starved condition. The amount of these biomolecules and heterocyst frequency was much higher when grown at 0.04 g/L $K_2HPO_4$ (control) (Table 4; Figs. 7 and 8). Thus, the role of phosphate in the growth and various growth parameters are established.

Temperatures above 20°C is responsible for the spontaneous inactivation of the enzyme by heat leading to diminished catalase activity [31]. Rady *et al*., [33] reported that *Synechocystis* PCC 6803 strain acclimated at different growth temperature exhibited significantly increased catalase activity at 20°C and decreased significantly when growth temperature was increased to 43°C. Studies have demonstrated the increased activity of antioxidant enzymes in response to increased light intensity to provide partial protection against oxidative stress in photoinhibitory condition [16,26]. *Nostoc muscorum* subjected to photoinhibitory light treatment showed increased antioxidant enzyme (SOD, catalase and peroxidase) activity [38]. In present study, temperature and light have acted as physical stressors leading to variation in catalase activity. High temperature (45$^o$C) has reduced the catalase activity in *F. thermalis*. Catalase activity has been higher in organisms incubated at 25$^o$C temperature (0.31 Unit $min^{-1}$ $g^{-1}$) as compared to higher temperatures (37$^o$C: 0.24 Unit $min^{-1}g^{-1}$; 45$^o$C: 0.18 Unit $min^{-1}$ $g^{-1}$). Similarly, high

light intensity (75 photons $m^{-2}$ $s^{-1}$ light) has been observed to be responsible for elevated expression of catalase activity (0.33 Unit $min^{-1}$ $g^{-1}$) over that in organisms incubated under 30 photons $m^{-2}$ $s^{-1}$ light (0.29 Unit $min^{-1}$ $g^{-1}$) in *F. thermalis*. Thus the decreased temperature and increased intensity of light may have acted as physical stressors in this particular cyanobacterium.

Thermophilic cyanobacteria have the potentiality to produce economically important and novel biocompound for commercial utilization in various field like pharmaceutical (carotenoids as antioxidant) and biocolourant (phycocyanin). But the optimization of growth parameters in culture condition is very essential for successful cultivation of these economically significant species. That is why major importance was given on optimization of cultivation technique of thermophilic cyabacteria. Experiments were conducted with the main goals to observe the effects of variation in composition of the growth medium on the morphology, physiology and metabolism of *F. thermalis*. Evidently varying concentrations of various growth parameters in BG-11 medium impart positive or negative impact on the morphology of cyanobacteria. This study signifies that the growth media for cultivation and culturing of thermophilic cyanobacteria should be modified in such a manner that the optimized yield of these biologically important microorganisms can be easily gained and no morphological abnormalities arise to hinder the proper and correct taxonomic identification of species.

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