RESEARCH ARTICLE

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Phosphatase activity of a thermos-halotolerant cyanobacterium: Effect of some environmental factors

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ABSTRACT

Cyanobacteria that grow above seawater salinity at temperatures above 45°C have rarely been studied. The present study is an attempt to decipher these unknown facts where the unique properties of phosphatase enzymes in a thermo-halotolerant Iceland clone 2 *Leptolyngbya* has been studied under some important environmental conditions that play a distinctive role in the growth of these extremophiles in those adverse ecological niches. *Leptolyngbya* used in this present study is a unique species having 2 extreme characteristics of tolerance to high salt concentrations and temperature, so it is of intrinsic and scientific interest to study the phosphate dynamics and its variability under different ecological factors. The results of these experiments clearly show that under very adverse conditions of low light or high temperature and very high salt concentrations (almost 3 times of salt present in seawater 90g/L) this extremophile has the capacity to maintain its growth and metabolism which is the key to its survival in these extreme habitats. Observations from growth experiments under different environmental conditions (Temperatures, pH, salt concentrations, different light intensities) under laboratory conditions were found to be like its diverse patterns and adaptive ability in the extreme environment this organism has been isolated from. Phosphatase activity as a way of understanding how P is metabolized under extreme conditions revealed that the highest phosphatase activity was observed in high salt concentrations (3 times that of seawater) and high temperatures of 45°C and low light intensities that is a very significant observation and scientifically important.

Key words: *Leptolyngbya*, phosphatase, extremophile, halophile.

Introduction

Organic phosphorus is a major component of the global phosphorus cycle and is made available for biological uptake through the action of phosphatase enzymes, which originate in the soil from plant root microorganisms (Healey, 1982; Wagner et al., 1995; Eberl et al., 1996; Clark et al., 1998; Banerjee, 2000a; Banerjee, 2000b; Banerjee, 2001; Whitton et al., 2005; Banerjee et al., 2007). If the supply of phosphate is sufficiently restricted so that an organism becomes phosphorus limited, one possibility to overcome this problem to use inorganic phosphate more efficiently (Mann, 1994). Cyanobacteria have evolved several strategies to deal with phosphate limitations. Most of the cyanobacteria are unable to take up large organic phosphate molecules but can cleave inorganic phosphate from a variety of substrates using extracellular alkaline phosphatase specifically (Whitton et al., 2005; Banerjee 2007). Cyanobacteria, like several other microorganisms, synthesize alkaline phosphatase enzymes in response to phosphorus deficiency in their external environment. (Bhaya et al., 2000; Hernández et al., 2002; Banerjee, 2007; Banerjee et al., 2007).

The uptake of phosphate by cyanobacteria has already been characterized in several cyanobacterial strains showing that the kinetic parameters for phosphate transport vary considerably among the cyanobacteria, depending also on the composition of the medium and the nutritional state of the cells (Marco & Orús, 1988; Thiel, 1988; Garbisu et al., 1993; Banerjee, 2007; Banerjee et al., 2007). Cultivation of cyanobacteria under P-limited conditions leads to higher rates of phosphate uptake (Thiel, 1988). Many cyanobacteria, to deal with P-limiting conditions, store polyphosphate reserves and induce the synthesis of extracellular phosphatases to obtain phosphate from organic substrates present in the surrounding medium (Wagner et al., 1995; Wagner & Falkner 2001; Banerjee & John, 2003).

Measurement of phosphatase activity of whole organisms or part of organisms is becoming a practical biochemical tool of biotechnological studies but there is a complete lack of knowledge in this area on extremophiles. The first report in this regard was on the Antarctic dry deserts by Banerjee et al., 2000 a, b. Regarding the phosphatase activity of thermophiles or halophiles, there is much left to be deciphered.

With the rapid development of the study of extremophiles, it is foreseeable that research in this field may drive the novel

area of science. It seems probable from fossil research that microorganisms, potentially cyanobacterial analogs to those that survive in the limits of life in the Antarctic dry deserts may have developed on Mars (Friedman et al.,1994, Wynn-Williams 2000, Banerjee et al., 2000; Banerjee & Sharma 2004, 2005). Other pioneering work on extremophilic organisms was carried out in the Negev deserts (Friedman et al., 1967).

Cyanobacteria are fundamentally important colonists of hot springs and hydrothermal vents. Their role in these extreme ecosystems is a result of their remarkable resistance to extreme temperature and high salinity whilst being capable of photosynthesis in adverse situations. Their production of compatible solutes makes them tolerate osmotic stresses resulting from desiccation, high temperature, and extreme salinity.

Numerous cyanobacteria are thermophiles (Ward & Castenholz, 2000) and many are known as halophiles, even as extreme halophiles (Oren, 2000), but there is little information on cyanobacteria that combine these 2 traits. Although there is initial molecular research on thermo-halophilic cyanobacterium *Leptolyngbya* from the dominant ecological niche and there is a paucity of knowledge regarding the behavior of this organism when isolated from its natural environment to lab conditions. The study of this organism, therefore, is not only important because it is biologically and scientifically very interesting, but because there is a great lacuna of knowledge regarding phosphate metabolism in this organism and which is essential for its growth and survival in the niche it belongs. Almost nothing is known about the mechanism adopted by this organism to perform phosphate metabolism under extreme conditions and how it supplements its phosphate requirement.

Thus, the study on the extremophile *Leptolyngbya* is important in evaluating the potential of this organism and other extremophiles under similar ecological niches to contribute to the phosphorus status of that niche. Apart from this, it is an intrinsic interest to study such organisms which survive on the borderline of life and sustain metabolic activities where their natural counterparts would perish. This investigation is most important before subjecting these organisms to any biotechnological applications.

Materials and Methods

Collection and identification of the organism

The culture of *Leptolyngbya* Iceland clone2 used in the present study, a thermo-halophilic extremophile was isolated from siliceous crusts at 40-45 degrees C in a geothermal seawater lagoon in southwest Iceland. Iceland Clone 2, *Leptolyngbya* morphotype, was selected for further study. This culture grew only at 45-50 degrees C, in a medium ranging from 28 to 94 g L(-1) TDS, After a detailed phylogenetic study on this strain with other similar strains by Professor (Dr.) Meenakshi Banerjee Department of Bioscience, Barkatullah University, Bhopal, India during her visit to Center of Ecology and Evolutionary Biology Oregon University, USA Iceland clone e *Leptolyngbya* was brought back to her laboratory of Algal Biotechnology for further work in India. Identifications had already been made in the USA by 16 S RNA analysis (Banerjee et al., 2009).

Culture media and culture conditions

The filaments (trichomes) were allowed to both migrate and grow out from the central source of inoculum on solid plates made with BG-11 medium and 15% Bacto Difco Agar. Minute agar blocks with an individual self-isolated trichome (clone) were removed with a sterile watchmaker's forceps under 40-609 magnification with a dissecting microscope and transferred into 125 mL capacity flasks of liquid IO BG-11 medium. After successful growth in flasks, the cultures were re-plated for a greater assurance of clonality. This Iceland clone 2 was morpho-logically identified with respect to 16S rDNA sequences and was chosen for the physiological experiments. All cultures were determined to be axenic, based on 1009 Nomarski examination for contaminating heterotrophic bacteria and by plating on agar medium containing 90% IO BG-11 dilute (10%) Plate Count Agar (Difco, Detroit, Michigan) that contains tryptone (5 g L-1), yeast extract (2.5 g L-1) and glucose (1 g L-1). These cultures were scaled up in BG-11 medium (Castenholz, 1988a, b), and with additions of NaCl as needed. Cultures of Iceland Clone 2e were maintained at 45 ± 1°C and illuminated continuously with cool white fluorescent lamps were maintained at 25 ºC±2º with a light flux of 2500-3000 Lux and light and dark rhythm of 16:8 hrs.

The cultures were grown for six months under the above-mentioned conditions to acclimatize the organisms to their new environment which is different from the environment they belong to before starting the experiment.

Growth measurement by chlorophyll analysis

The growth experiment was carried out in culture tubes each containing a 10 ml basal medium. The growth was measured by Chlorophyll extraction by the method described by Banerjee et al., 2000 and Marker, 1995. Chlorophyll was extracted in 10 ml of either 100% methanol or 80% acetone. The tubes were shaken for 5 min and then placed overnight in the dark, in a refrigerator to ensure complete extraction. The optical density of the extract was measured with a Systronic 169 spectrophotometer at 663 nm. The amount of Chlorophyll extracted was calculated according to the equation of Mackinney, 1941 (A = kc where A is the absorbance of light by the sample, k is a constant that depends on the solvent used,

and c is the concentration of chlorophyll in the sample). Here the algal chlorophyll was calculated as

Chlorophyll µg/ml = optical density x 12.63 x dilution factor.

The generation time (K) was calculated by growth equation of Kratz & Myers (1955).

$$K = 2.303 (\log N_2 - \log N_1)/ T_2 - T_1$$

Where, N_1= Initial OD / Protein concentration at time T_1, and N_2 = Final OD/ Protein concentration at time T_2.

Measurement of phosphatase enzyme activity

A study of alkaline phosphomonoesterase and phosphodiesterase of the Iceland clone was done by the method described by Mahasneh et al., 1990, Whitton et al., 1990 and Banerjee et al, 2007. PMEase (phosphomonoesterase) activity was routinely assayed by colorimetric method using para-nitro-phenyl-phosphate (p-NPP) and PDEase (phosphodiestrase) activity using bis-para-nitro-phenyl-phosphate (bis-p-NPP). Cyanobacterial cells were transferred to P-minus medium before the phosphatase assay for two weeks to deplete the cells of P. Assays were carried out in a P-free version of the medium and buffered with 100 µM glycine (final concentration) to give a pH of 8.0. Many of the practical details are given in Whitton et al., 1992. The results are expressed as µ mol (pNP) µg chl$a^{-1}h^{-1}$ and µ mol (bis-pNP) µg chl$a^{-1}h^{-1}$. All results mean ± standard deviation of three independent replicates.

Study of environmental factors

Effect of salt concentrations

The effect of different salt (NaCl) concentration from 30g.l^{-1} to 140g.l^{-1} on phosphatase activity (PMEase and PDEase) of *Leptolyngbya* was studied (Banerjee et al., 2009).

Effect of pH

The effect of pH from 5 to 10 on growth, PMEase, and PDEase activity of the organism was also studied. Different pH conditions were maintained with the help of a universal electrode pH meter Systronics Model No. 361 and the addition of 2 N NaOH and 1 N HCl according to the pH required. To avoid any changes in pH, medium was buffered with Tris (Hydroxy methyl) Methylamine/ HCl buffer Banerjee et al 2000, Whitton et al 1992.

Effect of temperature

The effect of different temperature ranges from 45ºC to 65ºC on growth, and phosphatase activity (PMEase and PDEase) of the organisms was also studied (Islam & Whitton, 1992). Experimental sets were kept in incubators set at temperatures for conducting the growth and phosphatase activity studies.

Effect of light intensity

For light intensity, the cultures were maintained in the culture room illuminated by three 150Watt fluorescent tubes. The light source was obtained from a cool fluorescent tube of about 150W. To avoid overheating, cultures were incubated in water-jacketed boxes. Variation in light intensity was obtained by increasing and decreasing the distance of cultures from the source of light using lux meter LX-101 Lutron, and the dark condition has been made by wrapping the culture tube or flasks twice with aluminum foil and carbon paper (Banerjee et al., 2000 a,b).

Statistical Analysis

Statistical analysis consisted of mean values and standard errors that are given in the figure and tables. Means were compared using student's t-test at $\alpha = 0.01$ and 0.05.

Results

Effect of Environmental factors on Growth

Figure 1 shows the generation time of *Leptolyngbya* tested in the three different media and the fastest growth was observed with BG-11 (N^+) medium compared to the other 2 mediums used. Figure 2 shows the effect of different NaCl concentrations from 30 g.l^{-1} to 140 g.l^{-1} supplemented in the medium to determine the survival and growth. Since the maximum growth was observed at 90 g.l^{-1} salinity, which is approximately three times more than seawater salinity (35 g.l^{-1}); this concentration was chosen as a control for further study on this organism. The control set was (BG-11 (N^+ medium without NaCl) in which a very slow growth was observed.

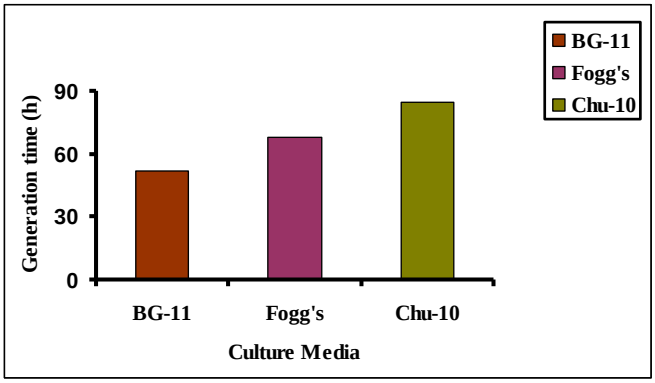

Figure 1. *Generation time of Leptolyngbya in three different culture media. Cultures grown under25°c± 2°c, light intensity 2500±200 lux, pH- 8.*

Temperature is one of the most important parameters for growth. The effects of different temperatures were studied on this strain. Figure 3 shows that maximum growth was observed at 45±2ºC (1.34 µg Chl *a* ml^{-1}) at 96 h and the fold increase was found to be 1.10 over control (25±2ºC) at 96 h. With the

increase in temperature i.e., 55±2ºC and 65±2ºC decrease in growth rate was observed. This was 0.56- and 0.52-fold decrease at 96 h over control at 55±2ºC and 65±2ºC, respectively.

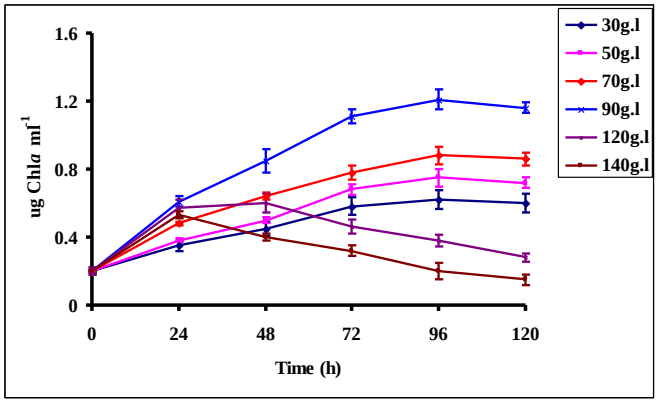

Figure 2. *Graph showing growth curve of Leptolyngbya in different salinities 25°c± 2°c, light intensity 2500±200 lux, pH- 8 (results mean ± SD, N=3).*

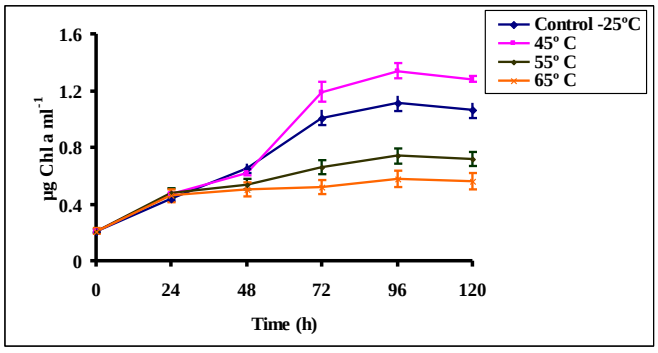

Figure 3. *Effect of different temperatures on growth of Leptolyngbya. light intensity 2500±200 lux, pH- 8 (results mean ± SD, N=3).*

The effects of different pH were also studied on growth, ranging from 5 to 10. During this study in Iceland clone *Leptolyngbya*, temperature (25±2ºC) and NaCl concentration (90 g.l^{-1}) were kept constant. Maximum growth was observed at pH-9 (1.38 µg Chl*a* ml^{-1}) at 96 h followed by pH-10 and control pH-8 at 96 h (Figure 4). In this cyanobacterium at pH-9 there was a fold increase of 1.14 over the control pH-8 at 96 h.

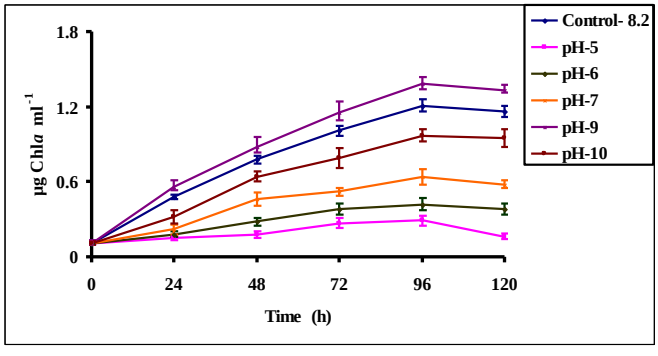

Figure 4. *Effect of different pH on growth of Leptolyngbya. temperature 25°c± 2°c, light intensity 2500±200 lux, (results are mean ± SD, N=3).*

Studies show that the growth of cyanobacteria is generally sensitive to light. Experiments with different light intensities on this cyanobacterium showed optimum growth at 2500±200 Lux light (1.21 µg Chl*a* ml^{-1}) under laboratory conditions at 96 h. With further increasing light intensities there was a decrease in growth (Figure 5A). At 5000±200 Lux light intensity, there was a fold decrease of 0.48 over control (2500±200 Lux light) at 96 h.

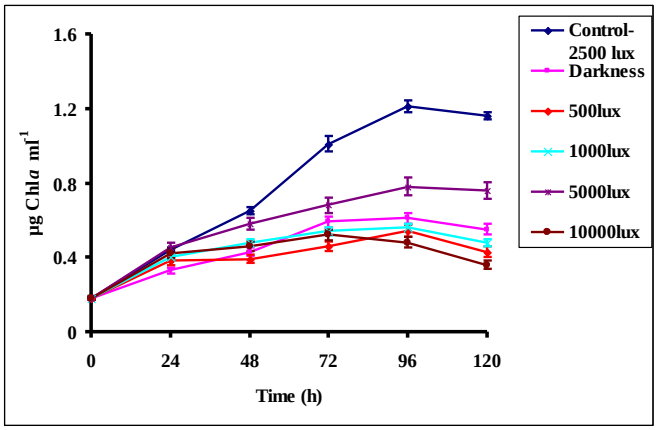

Figure 5A. *Effect of different light intensity in Leptolyngbya. Temperature 25°C± 2°C, pH- 8 (Results are Mean SD N=3).*

Figure 5B shows the effect of darkness on this organism under laboratory conditions. *Leptolyngbya* showed an increase in growth in complete dark condition (0.65 µg Chl*a* ml^{-1}) at 96 h compare to low light intensities i.e. 500±200 Lux light and 1000 Lux light. However, there was a 0.537-fold decrease over control (2500±200 Lux light) at 96 h.

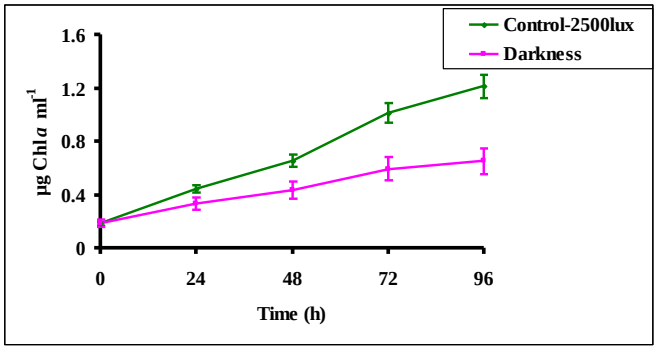

Figure 5B. *Effect of darkness on growth of Leptolyngbya . Temperature 25°c± 2°c, pH- 8 (results mean ± SD, N=3).*

Effect of environmental factors on phosphatase activity.

After obtaining results on growth in this Iceland strain *Leptolyngbya*, with different environmental factors the same conditions were chosen to study the effect on phosphatase activities. Like growth maximum phosphomonoesterase and phosphodiesterase activity were observed with 90g.L^{-1} of

NaCl therefore this concentration of NaCl was kept constant in further studies on phosphomonoesterase and phosphodiesterase activity (Figure 6A & B).

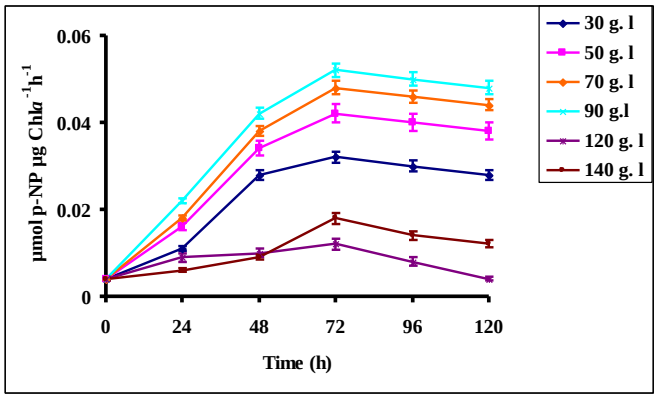

Figure 6A. *Effect of different salinity on phosphomonoesterase activity of Leptolyngbya. Temperature 25°c± 2°c, light intensity 2500±200 lux, pH- 8 (results mean ± SD, N=3).*

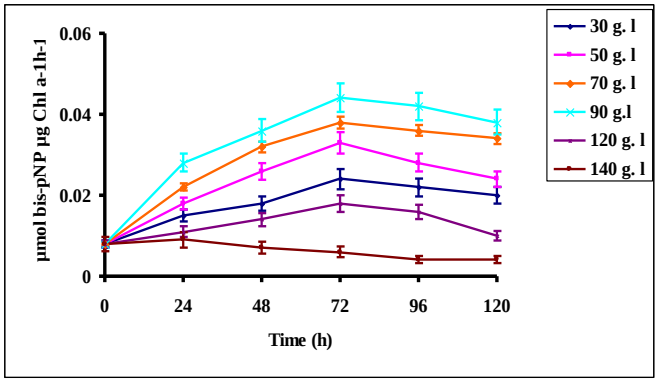

Figure 6B. *Effect of salinity on phosphodiesterase activity of Leptolyngbya. Temperature 25°c± 2°c, light intensity 2500±200 lux, pH- 8 (results mean ± SD, N=3).*

When the effect of different range of temperatures (45-65°C) on phosphatase activities were studied on *Leptolyngbya* maximum phosphomonoesterase activity was observed at 45±2ºC (0.058 µmol p-NP µg Chla^{-1}h^{-1}) at 72 h (Figure 7A) and the fold increase was found to be 1.11 over control (25±2ºC) at 72 h. With the increase in temperature i.e., 55±2ºC and 65±2ºC decrease in activity was observed. It was 0.92- and 0.88-fold decrease at 72 h over control at 55±2ºC and 65±2ºC respectively (Figure 7A). Maximum phosphodiesterase activity was also observed at 45±2ºC (0.046 µmol bis-p-NP µg Chla^{-1}h^{-1}) at 96 h after which the activity declined (Figure 7B). This was a 1.04-fold increase over control (25±2ºC) at 96 h. With increasing temperature i.e., 55±2ºC and 65±2ºC there was a gradual decrease in activity. It was a 0.86- and 0.81-fold decrease at 96h over control (25±2ºC) at 55±2ºC and 65±2ºC respectively (Figure 7A & B).

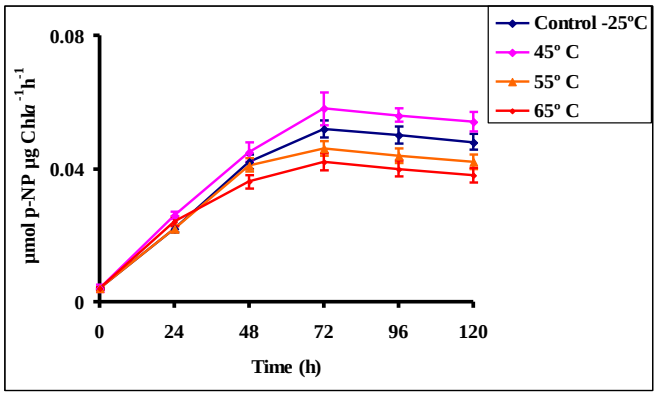

Figure 7A. *Effect of different temperatures on phosphomonoesterase of Leptolyngbya. Light intensity 2500±200 lux, pH- 8 (results mean ± SD N=3).*

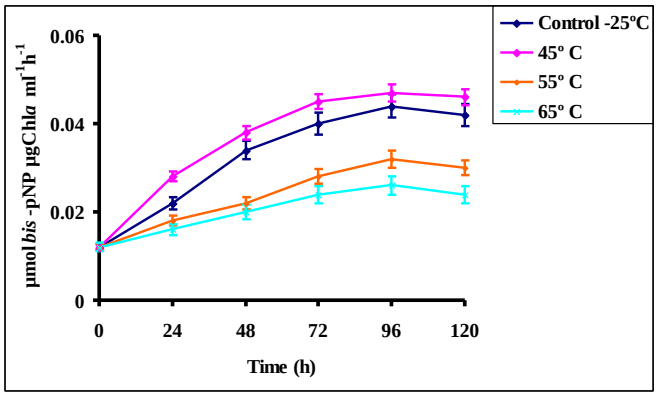

Figure 7B. *Effect of different temperatures on phosphodiesterase of Leptolyngbya. Light intensity 2500±200 lux, pH- 8 (results mean ± SD N=3).*

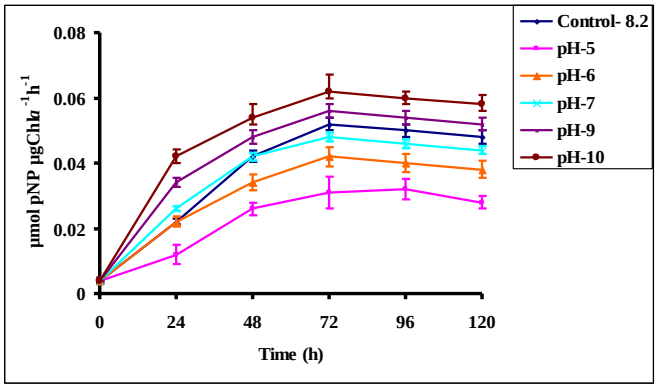

Figure 8A. *Effect of different pH on phosphomonoesterase activity of Leptolyngbya. Temperature 25°c± 2°c, light intensity 2500±200 lux. (Results mean ± SD, N=3).*

The effect of different pH on the phosphomonoesterase and phosphodiesterase activity were studied ranging from 5 to 10 on the strain. In *Leptolyngbya* maximum phosphomonoesterase activity was observed at pH-10 (0.062 µmol p-NP µg Chla^{-1}h^{-1}) at 72 h and followed by the pH-9 (0.058 µmol p-NP µg Chla^{-1}h^{-1}) at 72 h (Figure 8A). In this cyanobacterium at pH-10 there was a fold increase of 1.19 over the control pH-8 at 72 h (Figure 8B). Maximum

phosphodiesterase activity was observed at pH-9 (0.056 µmol bis-p-NP µg Chla^{-1}h^{-1}) at 96 h this was a 1.27-fold increase over the control pH-8 at 96 h (Fig. 5.5 b). The acidic pH 5, 6 and neutral 7 as given in (Figure 8A & B) failed to produce any significant impact on the phosphomonoesterase and phosphodiesterase activity of *Leptolyngbya* when compared to control (Figure 8A & B).

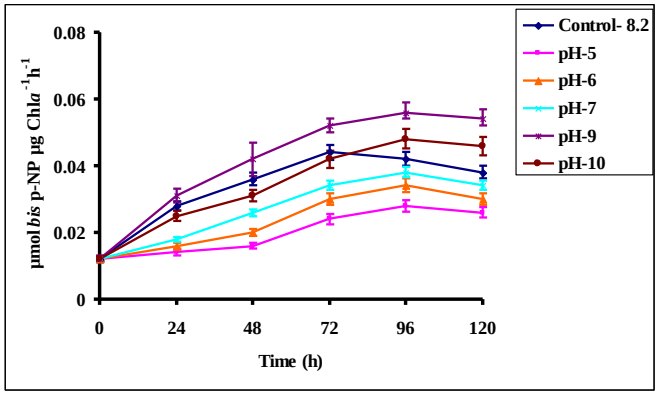

Figure 8B. *Effect of different pH on phosphodiesterase of Leptolyngbya. Temperature 25°c± 2°c, light intensity 2500±200 lux. (Results mean ± SD N=3).*

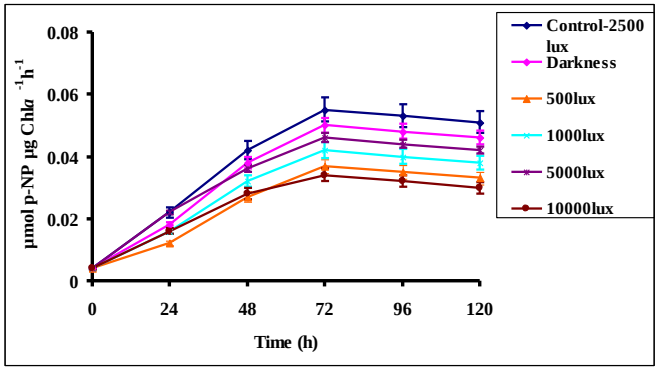

Figure 9A. *Effect of different light intensity on phosphomonoesterase activity of Leptolyngbya. Temperature 25°c± 2°c pH- 8 (results mean ± SD N=3).*

The effect of different light intensities was also studied on phosphomonoesterase and phosphodiesterase activity of the strain. Experiments with different light intensities on *Leptolyngbya* showed maximum phosphomonoesterase activity was observed at 2500±200 lux light (0.052 µmol p-NP µg Chla^{-1}h^{-1}) at 72 h (Figure 9A). The next best result was at 5000±200 Lux light (0.046 µmol p-NP µg Chla^{-1}h^{-1}). In *Leptolyngbya* with 5000±200 Lux light intensity, there was a fold decrease of 0.882 over control (2500±200 Lux light) at 72 h (Figure 9A). With other increasing light intensities there was a decrease in phosphomonoesterase activity observed at 72 h (Figure 9A). Phosphodiesterase activity was also observed to be highest at 2500±200 Lux light (0.044 µmol bis-p-NP µg Chla^{-1}h^{-1}) at 96 h. (Figure 9 B). With other increasing light intensities there was a decrease in phosphodiesterase activity at 96 h. Figures 10 A and B show the effect of darkness on the phosphomonoesterase and phosphodiesterase activity of the organism. *Leptolyngbya* showed a nominal decrease in phosphomonoesterase in dark conditions (0.050 µmol p-NP µg Chla^{-1}h^{-1}) at 72 h this was a 0.96-fold decrease compared to control light conditions (2500±200 lux light) at 72 h.

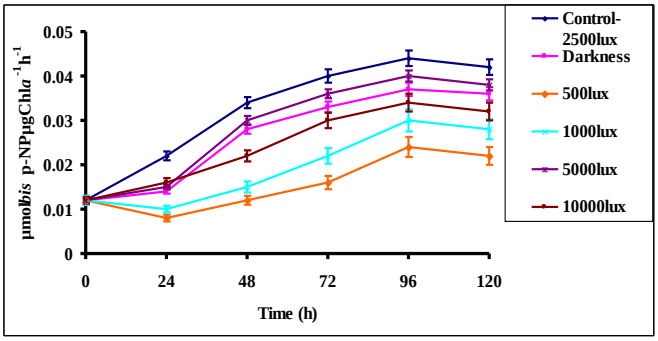

Figure 9B. *Effect of different light intensity on phosphodiesterase activity of Leptolyngbya. Temperature 25°c± 2°C, pH- 8 (results mean ± SD N=3).*

Discussion

In the present study, some significant observations were obtained which point to the variation in response of extremophilic *Leptolyngbya* when exposed to different environmental conditions. This is even more significant since there is little information on cyanobacteria that combine these 2 extreme traits like the Iceland clone 2 *Leptolyngbya*.

The members of the common and ecologically important cyanobacterial form–genus *Leptolyngbya* are widely distributed in numerous ecosystems all over the globe (Komarék, 1989). The result of the present study shows that *Leptolyngbya* can grow under a wide variety of extreme conditions too. The ability of *Leptolyngbya* to survive at a super higher NaCl concentration (90g.l^{-1}) is probably because of the adaptation to the conditions in the hypersaline thermal lake of Iceland where it was isolated. The significant growth in thrice the salt concentration of seawater can be explained by the fact that a considerable amount of Na^+ is required for the growth of halophilic cyanobacteria because it stimulates photosynthesis so rapidly, that HCO_3^- transport into the cell raises the intracellular CO_2 concentrations. In halophiles, adaptation to higher concentrations of NaCl is also dependent upon the osmotic adaptation of the organism, and organic solutes play an important role in osmotic adaptation.

Temperature is an important factor for growth in cyanobacteria. The strain under study can tolerate high temperatures of 45°C±2°C. Survival at high temperatures is an adaptation of halotolerant cyanobacteria because life in such hypersaline waters due to the high specific heat of brines, can easily reach elevated temperatures with sunlight. In halophiles, temperature causes increased cell density but above a certain optimum temperature cell density gets reduced by adversely

affecting the chlorophyll and carotenoid pigments. The pH of an ecological niche is the most important factor that determines the cyanobacterial occurrence, which is reported from a wide range of acidic to alkaline environments. The pH optimum for the growth of *Leptolyngbya* was 9. At alkaline pH, a large number of cations such as Na^{+}, Mg^{++}, Ca^{++}etc. which are taken up by the organism and thus increase the photosynthetic efficiency of cells. The negligible growth at low pH proves that acidic medium is detrimental to the growth of the strain. Acidic pH might affect the photosynthetic apparatus, moreover, chlorophyll *a* is very acid labile and decomposes to pheophytin under acidic conditions.

Light availability influences cyanobacterial growth. The observed effects in this study show that the growth of cyanobacteria is generally sensitive to high light intensities. In halophiles, high light intensity may reduce the functioning of the reaction center of PS I and PS II therefore the observed decrease in growth of *Leptolyngbya* at high intensity. Laboratory experiments using white light have generally indicated that at very low irradiance intensities cyanobacteria have low light requirements for growth. Natural hot spring mats suggest that many cells making up the algal layer might not become light-saturated even at high light intensities because of self-shading, except for some thermophilic microorganisms that avoid high light irradiance by active gliding motility response causing a move downward into the soft microbial mats 'or' sediments with increasing irradiance, thereby avoiding the stress entirely. The ratio of chlorophyll *a* to phycobiliprotein remained constant during light-limited growth, despite the reduction in pigments as irradiance increased. This indicates that the number of photosynthetic units per cell declines in response to increasing irradiance. Light is an important factor for the growth and photosynthesis process and is clearly demonstrated in this study.

The slight decrease in growth rates in the dark is related to the non-availability of reductant and energy in the form of ATP, due to which the photosynthetic process is affected which leads to a decrease in the growth rate of the organism compared to light conditions.

Cyanobacteria can synthesize phosphates in response to phosphorus deficiency in their external environment. Phosphate-solubilizing microorganisms produce extracellular enzymes like phosphatases along with organic acids for phosphate solubilization. Cyanobacteria secrete alkaline phosphatase into the surrounding medium under phosphate-starved conditions. Cyanobacteria during a long period of P-limitation excrete extracellular phosphatases, which frequently release inorganic phosphate (Pi) from a few complex organic phosphate substrates (Stihl et al., 2001) that cannot be transported through the cell membrane. Alkaline phosphatase is the first enzyme, and it remains active in a free dissolved state in natural water.

The high alkaline phosphatase activity that was obtained in a saline medium in the present study indicates halotolerance of phosphatase enzyme in *Leptolyngbya*. In the natural environment, *Leptolyngbya* is found in salinity thrice that of seawater and this coincides with the laboratory-grown cells (90 $g.l^{-1}$). NaCl stress at lower concentrations might be caused by an increase in cellular PMEase activity, while a higher concentration of NaCl might be favored release of the extracellular PMEase. An increase in activity in higher concentrations of NaCl (90$g.l^{-1}$) suggests that the higher concentrations of salt stimulate the release of the enzyme. The release of cell-bound enzymes into the medium proves that alkaline phosphatases are loosely bound to structural elements of the cell wall and can be easily released from cells by osmotic shock. NaCl has a concentration-dependent dual effect on phosphatase activity in this strain. The results suggested that phosphomonoesterase and phosphodiesterase might be inducible enzymes in this organism. This study also suggests that high salinity seems to trigger phosphate uptake there for providing the necessary energy in the form of ATP resulting marked increase in alkaline phosphatase activity observed.

In this study, significantly less PDEase activity compared to PMEase activity is observed. This is probably because the PDEase releases about 25% of the inorganic phosphate from bis-pNPP compared to that released by pNPP. The monoesters can provide the necessary levels of phosphorus to the cells as substrates and are easily available compared to diesterases and their activity.

Cyanobacteria have an inherent capacity to respond to environmental changes by adapting themselves. Temperature is considered one of the most important environmental factors controlling the enzyme activity and metabolism of the organism (Sigee, 2005). The results obtained from the present study suggest that the temperature at which the alkaline phosphatase activity was maximum was almost the same as the hot spring temperature from where the organisms have been isolated. Thus, it becomes a matter of intense scientific interest to further use this thermotolerant phosphatase enzyme for industrial exploitation as a big application of this study. It also indicates that even in very harsh conditions of very high-temperature phosphatase contributes to the P availability and economy in that ecological niche.

The pH values which gave maximum growth and phosphatase activity were quite close to those reported for most cyanobacteria and this also coincides with the pH conditions present in the hot springs. Thermophilic cyanobacteria occur in environments with pH values of 7.0-9.5. Na^{+} is also known to be involved in regulating the internal pH of the cell in cyanobacteria. The negligible rates of alkaline phosphatase activity at low pH i.e., 5-7 proved that the acidic medium is detrimental to alkaline phosphatase activities as this

enzyme gets induced well above pH-7 and which goes in accordance with the name of the enzyme alkaline phosphatase.

The energy dependence of the phosphate uptake process was observed in different light intensities and dark conditions. It might be expected that the activity of an enzyme occurring at the cell surface would not be closely linked to photon irradiance, at least in the short term. In the present study, it has been observed that light is likely to enhance phosphate uptake from the vicinity of the enzyme and thus reduce possible substrate inhibition. This suggests that light had a positive effect on phosphates activity.

Approximately similar enzyme activity in light and dark condition suggests that although there is a distinct light regime in hot spring, fluctuations and decrease in light intensity may not affect phosphatase activity in extremophilic cyanobacteria. Apart from that in its natural environment, these cyanobacteria are found in layers this ability allows not only the naturally occurring organism but also the laboratory-grown cultures under dark conditions. The almost similar effect of light and darkness on PMEase and PDEase activities was unexpected. Much of the effect appeared to occur during the early stage of assays so the percentage decrease dropped during long-term assays. Nevertheless, this effect was consistent and repeatable, and there was no obvious source of an artifact. Most studies on surface phosphatases show no effect of light during short-term studies, though a few studies on seaweeds have shown enhancement in the light (Hernandez et al., 2000), where the effect is thought to reflect the demand for energy to transport the phosphate released by hydrolysis into the cell. The red alga *Corallina elongata* is apparently the only organism reported to show slightly higher activity in the dark than under low light flux (Hernandez et al., 1996). It was suggested that phosphatase activity may help fulfill P requirements in this organism when direct uptake of inorganic P is reduced under conditions of limited reducing power. This might apply to the Iceland clone *Leptolyngbya* and possibly the surface phosphatase(s) in this organism undergoes some form of change on transfer from dark to light. Any factor that affects the metabolism of phosphates, which is crucial for biomolecules associated with energy exchange and genetic processes at the limits of survival, is of great importance.

Conclusion

The presence of significant phosphatase activity in the Iceland clone 2 *Leptolyngbya* strain despite its exposure to high levels of salinity and high temperature suggests that this cyanobacterium does not lose the capacity to form phosphatase enzymes even under those adverse conditions. The presence of cyanobacterial phosphatases may have very important ecological implications in diverse ecological niches including the extremophilic environments where there is a constant fluctuation in nutrients, especially of phosphate, and organic P may be a frequent alternative to inorganic P as these cyanobacteria are the dominant organisms in the algal/cyanobacterial flora in these environments. Significant enzyme activity in both light and dark for *Leptolyngbya* implies that phosphatase activity may not be affected by changes in light regimes in thermophilic environments. Cyanobacterial cells within the immobilized mats of cells on the boundary of the thermal lake from where the cyanobacterium was isolated could become light-limited by the shading effect in the lower layers but even under these conditions results predict that phosphatase activity is not affected by limiting light conditions. Observations of the present study suggest that in other similar extreme ecological niches, alkaline phosphatase activity can contribute significantly to maintaining the growth of cyanobacteria for prolonged periods even under unfavorable conditions of phosphorus depletion. This Iceland strain with 2 extreme traits has a thermohalotolerant phosphatase enzyme making it extremely important for industrial applications.

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