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INFLUENCE OF TEMPERATURE ON ALKALINE PHOSPHATASE ACTIVITY IN CYANOBACTERIA (BLUE-GREEN ALGAE)

V.D. Pandey
Department of Botany, Pt. L.M.S. Campus, Sridev Suman Uttarakhand University
Rishikesh-249201, Dehradun, Uttarakhand, India; E-mail: pandeyvidya2@gmail.com

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ABSTRACT

The enzyme alkaline phosphatase (APase), which catalyzes the degradation of complex organic phosphate substrates into organic moiety and biologically available inorganic phosphate (Pi), plays an important role in the growth and survival of cyanobacteria under the conditions of phosphorous deficiency in the ambient environment. APase activity serves as an indicator of phosphorus status in phytoplanktons. Various physicochemical factors can influence alkaline phosphatase activity in cyanobacteria. The present study was undertaken to investigate the influence of varying temperature in the range 10-50 °C on alkaline phosphatase (cellular and extracellular PMEase) activity in two freshwater cyanobacteria *Synechocystis* sp. (unicellular form) and *Oscillatoria* sp. (filamentous non-heterocystous form). In both the cyanobacteria, cellular and extracellular PMEase were observed to be maximally active at temperature 35°C and 40°C, respectively. The activity of cellular PMEase increased with the increase in temperature from 10ºC to 35ºC and thereafter decreased abruptly. The activity of extracellular PMEase increased up to 40ºC followed by a drastic decrease beyond 40ºC. The results of the study indicate that temperature is an important environmental factor influencing cyanobacterial APase activity.

Keywords: Cyanobacteria, Phytoplanktons Alkaline phosphatase, Phosphomonoesterase, Phosphorus.

INTRODUCTION

Cyanobacteria (Blue-green algae) are a morphologically diverse and widely distributed group of photosynthetic gram-negative prokaryotes which exhibit oxygenic photosynthesis similar to plants (Fogg *et al*., 1973; Stanier and Cohen-Bazire, 1977). Morphologically, they range from simple unicellular and coccoid to complex filamentous forms. Cyanobacteria are ecologically versatile, highly adaptable and widely distributed organisms, occurring or growing in various terrestrial and aquatic habitats situated at all latitudes from tropical to polar (Tandeau de Marsac and Houmard, 1993; Whitton and Potts, 2000). In natural environments, they grow as free-living organisms, in symbiotic associations with plants (e.g. fungi, bryophytes, pteridophytes, gymnosperms and angiosperms) and invertebrate animals (e.g. corals, sponges, and hydroids), and in microbial mats (Stal, 1995; Adams, 2000; Rai et al., 2000). Both ecologically and economically, cyanobacteria are recognized as an important organism. In ecosystem, they play prominent role in carbon, oxygen and nitrogen cycling (Tomitani *et al.*, 2006; Waterbury *et al.*, 1979). As photosynthetic organisms, they are important primary producers, and contribute significantly to the primary production of various ecosystems, particularly freshwater and marine ecosystems. It is estimated that they account for about 30% of global primary production (Hagemann, 2011). A large number of cyanobacteria are planktonic, occurring in aquatic (freshwater and marine) ecosystems. Planktonic cyanobacteria include unicellular, colonial, non-heterocystous and heterocystous filamentous forms with a wide range of size- picoplanktonic (<5 µm), nanoplanktonic (5-20 µm) and microplanktonic (>20 µm) (Paerl, 2000).

Among various physical and chemical factors, the availability of phosphorus (P) greatly influences the growth, development and population dynamics of phytoplanktons in freshwater environments (Oliver and Ganf, 2000; Sigee,2005). Phosphorus is an essential

element for all organisms, including cyanobacteria as it is a component of biomolecules like nucleic acids and phospholipids, and is involved in energy transformation (ATP). In cyanobacteria, phosphorus deprivation causes marked physiological or biochemical changes, such as reduction of growth, chlorosis, reduction in cellular constituents (e.g. proteins, nucleic acids, photosynthetic pigments, ATP), degradation of phycobilisomes and accumulation of cyanophycin granules (Block and Grossman, 1988; Stevens *et al.,* 1981).

Assimilation of phosphorus by cyanobacteria and other phytoplanktons is restricted to uptake of phosphate ions (PO_4^{3-}), which constitute the biologically available phosphorus (Sigee, 2005). Surplus phosphorus is stored by cyanobacterial cells in the form of insoluble polyphosphate, which is utilized for cellular metabolism during short periods of phosphorus starvation (Bhaya *et al.,*2000). However, during long periods of phosphorus deprivation or limitation cyanobacteria secrete extracellular alkaline phosphatase (APase) which catalyzes the degradation of various complex organic phosphate substrates into organic moiety and biologically available inorganic phosphate (Pi) (Stihl *et al.,*2001). The ability to utilize various organic P-substrates is common in cyanobacteria (Whitton *et al*., 1991). APase, a zinc containing metallo-enzyme, shows maximum activity at alkaline pH. Alkaline phosphatase which catalyzes the enzymatic breakdown of monoester bonds (in phosphomonoesters) is referred to as phosphomonoesterase (PMEase) whereas that acts on diester bonds (in phosphodiesters) as phosphodiesterase (PDEase). The activity of APase constitutes one of the survival strategies in cyanobacteria to grow and survive under conditions of phosphate deficiency (Bhaya *et al.*, 2000). APase activity serves as a simple and effective indicator for phosphorus status of phytoplanktons (Yoshimura and Kudo,2001). Various physicochemical factors are known to influence alkaline phosphatase activity in cyanobacteria (Singh *et al*., 2006; Singh and Tiwari, 2000; Li *et al*., 2013). Temperature is an important physical factor which strongly influences growth and various physiological and biochemical processes in cyanobacteria (Inoue *et al*., 2001; Murata, 1989; Shukla and Kashyap, 1999).

The present study was undertaken to study the influence of varying temperature (in the range 10-50 °C) on alkaline phosphatase (cellular and extracellular PMEase) activity in freshwater cyanobacteria *Synechocystis* sp. (unicellular form) and *Oscillatoria* sp. (filamentous non-heterocystous form).

MATERIALS AND METHODS

Cyanobacteria were sampled from a local pond in Rishikesh (30º06'25"N- 78º17'56" E), Uttarakhand (India), using wide-mouthed screw-cap tubes. They were identified microscopically based on morphological characteristics (nature, shape and dimensions of cells, colonies and filaments; presence/absence and position of heterocysts and akinetes; shape of intercalary and end cells; presence/absence and pattern of sheaths; polarity) with the help of standard literature (Desikachary, 1959; Rippka *et al*., 1979). They were established as clonal (unicyanobacterial) and axenic cultures by plating/streaking, repeated sub-culturing on solidified and in liquid medium, and antibiotic treatment following standard microbiological methods/techniques as described by Rippka (1988). They were grown photoautotrophically in sterilized BG-11 culture medium (Rippka *et al.,* 1979) in cotton-stoppered Erlenmeyer flasks at 26±2°C and under continuous illumination (light intensity at the surface of culture flasks, 1.5 Klux PAR) provided by cool-white fluorescent tubes. The pH of the medium was maintained at 7.6 with HEPES buffer (2 mM). The total protein content of cultures was measured following the method of Lowry *et al*. (1951) as modified by Herbert *et al*. (1971).

Cyanobacterial cells growing exponentially in the basal medium (BG-11 medium) containing 40 mg l^{-1} phosphate were harvested by centrifugation (5,000xg, 10 min). After thorough washing of harvested cells in distilled water, they were transferred to the phosphate deficient medium (-Phosphate) and incubated for 24 h. Phosphate depleted cells were analyzed for alkaline phosphatase (PMEase) activity following the method as described by Ihlenfeldt and Gibson (1975) in glycine-NaOH buffer (pH 10.2) using p-nitrophenyl phosphate (p-NPP) as substrate. The extracellular PMEase activity was determined in the culture filtrates. The reaction mixtures containing 0.2 ml of culture suspension (for cellular PMEase)/culture filtrates (for extracellular PMEase), 1.6 ml of glycine-NaOH buffer (0.2 M) and 0.2 ml of p-NPP (final concentration of 0.75 mM) were incubated for 20 min at 37ºC in a temperature-controlled water bath. The activity was terminated by the addition of 8.0 ml of NaOH (0.2 M). The yellow colour developed because of enzymatic activity was quantified spectrophotometrically at 410 nm using p-nitrophenol as the standard. The enzymatic activity was expressed as nmol p-NPP hydrolyzed mg $protein^{-1}h^{-1}$. To study the effect of temperature on APase activity, homogenous

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cultures/culture filtrates of cyanobacteria were pre-incubated for 60 min under photoautotrophic growth conditions at varying temperature in the range 10-50 °C before measuring the enzyme activity. The desired temperature was achieved in a BOD incubator fitted with fluorescent tubes. The results were expressed as mean (±SD) of three independent replicates.

RESULTS

The influence of varying temperature ranging from 10 ºC to 50ºC on alkaline phosphatase (cellular and extracellular PMEase) activity was investigated at pH 10.2 in freshwater cyanobacterial species *Synechocystis* sp. and *Oscillatoria* sp. The assessment of alkaline phosphatase (APase) activity in both the cyanobacteria revealed marked variation in cellular and extracellular phosphomonoesterase (PMEase) activity. The result of the influence of temperature on APase activity (cellular and extracellular PMEase) in cyanobacteria *Synechocystis* sp. (unicellular form) and *Oscillatoria* sp. (filamentous non-heterocystous form) is presented in Fig. 1. The activity cellular and extracellular PMEase in both the cyanobacteria was found to be maximum at temperature 35ºC and 40ºC, respectively. In both the cyanobacterial species, the activity of cellular PMEase increased with the increase in temperature from 10 °C to 35 ˚C, and thereafter decreased abruptly. The activity of extracellular PMEase increased up to 40ºC followed by drastic decrease beyond 40ºC. As compared to the maximum activity of cellular PMEase recorded at 35 ˚C in both the cyanobacterial species, there was 54.6 % and 49.38 % decrease in its activity in *Synechocystis* sp. and *Oscillatoria* sp., respectively at 50 ˚C, the maximum temperature tested. Similarly, as compared to the maximum activity of extracellular PMEase recorded at 40ºC in both the cyanobacterial species, there was 82.8 % and 70.52 % decrease in its activity in *Synechocystis* sp. and *Oscillatoria* sp., respectively at 50 ˚C,

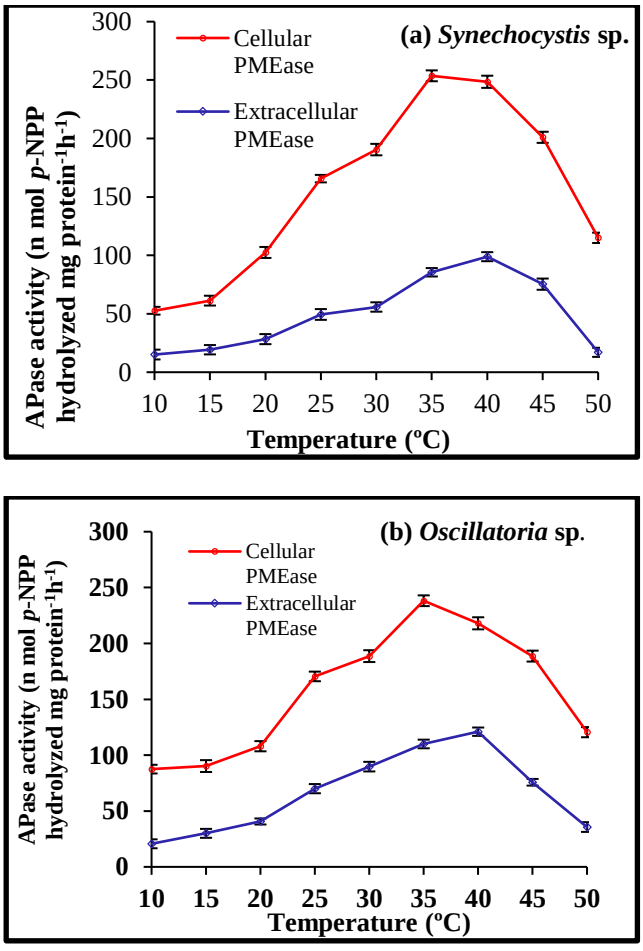

Fig.1. Influence of temperature on alkaline phosphatase (cellular and extracellular PMEase) activity in cyanobacteria (a) *Synechocystis* sp. and (b) *Oscillatoria* sp. Values are mean ± SD (n=3). Bars denote SD.

DISCUSSION

Cyanobacteria are important biotic components of aquatic ecosystems. As a major limiting nutrient, biologically available phosphorus in form inorganic phosphate is often present as a scarce resource in freshwater environments. APase plays a crucial role in phosphorus metabolism and regulation in cyanobacteria. The synthesis of APase by cyanobacteria is one of the adaptive strategies to cope up with phosphorus-deficient conditions. APase confer advantage to producing organism in competition for resources in a situation when internal reserves or ambient Pi is scarce. APase is a repressible-derepressible enzyme system regulated by the intracellular phosphorus content which ultimately depends on the external phosphorus concentration. When cyanobacteria and other microorganisms are cultured in Pi-deficient medium, the synthesis of APase is derepressed (induced). APase activity in cyanobacteria is influenced not only by the type and concentration of the substrate but also by various factors,

such as temperature, light/radiation, pH, nutrients (macro and micronutrients), salinity and the presence of metal ions (Singh *et al*., 2006; Singh and Tiwari, 2000; Li *et al*., 2013). Often, an inverse relationship exists between the activity of alkaline phosphatase and the concentration of orthophosphate in the ambient aquatic environment. Enzymes constitute one of the temperature-sensitive systems in cyanobacteria and other living organisms. Every enzyme has optimum temperature at which it shows maximum activity. In the present study, cellular and extracellular PMEase in the investigated cyanobacteria were found to be optimally active at temperature 35 °C and 40 ˚C, respectively. However, the optimum temperature for the growth of cyanobacteria, which mostly ranges between 25 ˚C and 30 ˚°C, has no bearing with the temperature optima for the APase activity. The effect of temperature on APase activity, as observed in present investigation, is consistent with the previous reports on cyanobacteria (Singh et al. 2006; Li et al. 2013). The synthesis and activity of alkaline phosphatase in cyanobacteria, in addition to physiological significance, have ecological significance. The cyanobacterial species with inducible alkaline phosphatases can be employed in biomonitoring of phosphorus status of aquatic ecosystems.

REFERENCES

Adams DG (2000). Symbiotic interactions. In: Whitton BA and Potts M (eds.) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 523-561.

Bhaya D, Schwarz R and Grossman AR (2000). Molecular responses to environmental stress. In: Whitton BA and Potts M (eds.) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 397-442.

Block MA and Grossman AR. (1988). Identification and purification of a depressible alkaline phosphatase from *Anacystis nidulans* R2. Plant Physiol. 86: 1179-1184.

Desikachary TV (1959). Cyanophyta. Indian Council of Agricultural research, New Delhi.

Fogg GE, Stewart WDP, Fay P and Walsby AE (1973). The Blue-green algae. Academic Press, London and New York.

Hagemann M (2011). Molecular biology of cyanobacterial salt acclimation. FEMS Microbiol Rev. 35: 87-123.

Herbert D, Phipps PJ and Strange RE (1971). Chemical analysis of microbial cells. In: Norris JR and Ribbons DW(eds). Methods in Microbiology, Vol. VB, Academic Press, London and New York, pp. 209-344.

Ihlenfeldt MJA and Gibson J (1975). Phosphate utilization and alkaline phosphatase activity in *Anacystis nidulans* (Synechococcus). Arch. Microbiol. 102: 23-28.

Inoue N, Taira Y, Emi T, Yamane Y, Kashino Y, Koike H *et al.* (2001). Acclimation to the growth temperature and the high temperature effects on photosystem II and plasma membranes in a mesophilic cyanobacterium *Synechocystis* sp. PCC6803. Plant Cell Physiol. 42: 1140-1148.

Li P, Liu W and Gao K (2013) Effects of temperature, pH, and UV radiation on alkaline phosphatase activity in the terrestrial cyanobacterium *Nostoc flagelliforme* . *J Appl Phycol* 25: 1031–1038. https://doi.org/10.1007/s10811-012-9936-8.

Lowry OH, Rosenbrough NJ, Farr AL and Ranall RJ (1951). Protein measurement with the folin phenol reagent. J. Biol. Chem. 193: 265-275.

Murata N (1989). Low-temperature effects on cyanobacterial membranes. J. Bioenerg. Biomembr. 21: 61-65.

Oliver RL and Ganf GG (2000). Freshwater Blooms. In: Whitton BA and Potts M (eds.) the ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht. The Netherlands, pp. 149-194.

Paerl HW (2000). Marine Plankton. In: Whitton BA and Potts M (eds.) The ecology of cyanobacteria: their diversity in time and space, Kluwer Academic Publishers, Dordrecht, The Netherlands. pp. 121-148.

Rai AN, Soderback E and Bergman B (2000). Cyanobacteria-plant symbioses. New Phytol.147: 449-481.

Rippka R (1988) Isolation and purification of cyanobacteria. In: Packer L and Glazer AN (eds.). Methods in Enzymology 167: 3-27.

Rippka R, Deruelles J, Waterbury JB, Herdman M and Stanier RY (1979). Generic assignments, strain histories and properties of pure cultures of cyanobacteria. J. Gen. Microbiol. 111: 1-61.

Shukla SP and Kashyap AK (1999). The thermal responses and activation energy of PS II, nitrate uptake and nitrate reductase activities of two geographically different isolates of *Anabaena.* Cytobios 99: 7-17.

Sigee DC (2005). Freshwater Microbiology: Biodiversity and Dyanamic interactions of Microorganisms in the Aquatic Environment. John Wiley & Sons, Ltd., Hoboken, USA.pp.235-286.

Singh SK and Tiwari DN (2000). Control of alkaline phosphatase activity in *Anabaena oryzae* Fritsch. J Plant Physiol 157: 467–472.

Singh SK, Singh SS, Pandey VD and Mishra AK (2006) Factors modulating alkaline phosphatase activity in the diazotrophic rice field cyanobacterium, *Anabaena oryzae*. World J Microbiol Biotechnol 22: 927-935.

Stal LJ (1995). Physiological ecology of cyanobacteria in microbial mats and other communities. New Phytol.131: 1-32.

Stanier RY and Cohen-Bazire G (1977). Phototrophic prokaryotes: the cyanobacteria. Ann. Rev.Microbiol.31: 225-274.

Stevens SE, Paone DAM and Balkwill DL (1981). Accumulation of cyanophycin granules as a result of phosphate limitation in *Agmenellum quadruplicatum*. Plant Physiol. 67: 716-719.

Stihl A, Sommer U and Post AF (2001). Alkaline phosphatase activities among populations of the colony-forming diazotrophic cyanobacterium *Trichodesmium* sp. (cyanobacteria) in the red sea. J Phycol 37: 310-317.

Tandeau de Marsac N and Houmard J (1993). Adaptation of cyanobacteria to environmental stimuli: new steps towards molecular mechanisms. FEMS Microbiol Rev.104: 119-190.

Tomitani A, Knoll AH, Cavanaugh CM and Ohno T (2006). The evolutionary diversification of cyanobacteria: molecular-phylogenetic and paleontological perspectives. PNAS. 103(14):5442-5447.

Waterbury JB, Watson SW, Guillard RRL and Brand LE (1979). Widespread occurrence of a unicellular, marine, planktonic cyanobacterium. Nature 227: 293-294.

Whitton BA and Potts M (2000). Introduction to the cyanobacteria. In: Whitton BA and Potts M (eds.) The ecology of cyanobacteria: their diversity in time and space. Kluwer Academic Publishers, Dordrecht. The Netherlands, pp. 1-11.

Whitton BA, Grainger SLJ, Hawley GRW and Simon JW (1991). Cell-bound and extracellular phosphatase activities of cyanobacterial isolates. Microbial Ecol. 21: 85-98.

Yoshimura T and Kudo I (2001). Seasonal variations in nutrients and a factor limiting phytoplankton growth in lake Ohnuma. Jpn J Limnol 62:205-217.