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Review Article

ALKALINE PHOSPHATASE ACTIVITY IN CYANOBACTERIA: PHYSIOLOGICAL AND ECOLOGICAL SIGNIFICANCE

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ABSTRACT
Cyanobacteria (blue-green algae) are a large and morphologically diverse group of oxygenic photosynthetic prokaryotes that possess remarkable adaptability to varying environmental conditions. They successfully colonise almost all kinds of terrestrial and aquatic ecosystems, including extreme and polluted environments. Planktonic cyanobacteria are ecologically important components of both marine and freshwater ecosystems, as they contribute significantly to the primary production of these ecosystems. Among various physical and chemical factors, the availability of phosphorus (P) greatly influences the growth, development and population dynamics of phytoplanktons in aquatic environments. Phosphorus is an essential element for all organisms, including cyanobacteria. As a major limiting nutrient, phosphorus is often present as a scarce resource in freshwater environments. It occurs in freshwater bodies as soluble organic, insoluble organic (biota and detritus) and soluble inorganic (phosphate) forms. Like other phytoplanktons, assimilation of phosphorus by cyanobacteria is restricted to uptake of phosphate, which constitutes the biologically available phosphorus. However, during long periods of phosphorus deprivation or limitation cyanobacteria produce and excrete extracellular alkaline phosphatase (APase) which catalyses the degradation of various complex organic phosphate substrates into organic moiety and biologically available inorganic phosphate (Pi) at alkaline pH. APase, a zinc containing metallo-enzyme, shows maximum activity at alkaline pH. Its production and activity constitute a survival strategy of cyanobacteria to grow and survive under conditions of phosphorus deficiency. APase activity serves as a simple and effective indicator for phosphorus status of phytoplanktons. The extracellular APase remains active in dissolved state in natural waters where it is believed to play role in nutrient dynamics.

Key Words: *Cyanobacteria, Alkaline phosphatase, Phosphomonoesterase, Phytoplanktons, Phosphorus*

INTRODUCTION
Cyanobacteria (blue-green algae) are a morphologically diverse and widely distributed group of photoautotrophic or photosynthetic gram-negative prokaryotes which exhibit oxygenic (O_2-evolving) photosynthesis similar to plants (Fogg *et al*., 1973; Stanier and Cohen-Bazire, 1977). They are among the primitive or oldest life forms on earth which, according to the recent study, evolved around 2.4 billion years ago (Fischer, 2008). They were the first organisms to evolve oxygen in Earth's atmosphere (Castenholz and Waterbury, 1989; Tandeau de Marsac and Houmard, 1993). Because of their oxygenic photosynthesis, they are believed to be responsible for the conversion of the primitive anaerobic reducing environment to aerobic oxidizing environment (Fischer, 2008). They have played a major role in cellular evolution by converting the primitive earth from anaerobic to aerobic (Margulis, 1975). In nature, they grow as free-living organisms, in symbiotic associations with a wide range of lower and higher plants, and in microbial mats (Stal, 1995; Adams, 2000; Rai et al., 2000). They interact with diverse microorganisms, such as heterotrophic bacteria, fungi, protozoans and viruses (Whitton, 1973). Cyanobacteria are ecologically and economically important organisms. Ecologically, they contribute significantly to the primary production of various ecosystems, especially freshwater and marine ecosystems, and play significant role in carbon, oxygen and nitrogen cycling (Tomitani *et al.*, 2006; Waterbury *et al.*, 1979). The biotechnological applications of cyanobacteria in diverse areas, such as bioremediation and pollution control, bioenergy and biofuels, nutraceuticals and pharmaceuticals have been well-documented (Patterson, 1996; Abed *et al*., 2009; De Phillips et al., 2003; Pandey, 2010). Owing to their remarkable adaptability to varying environmental conditions, they successfully colonize almost all kinds of terrestrial (agricultural soils, grassland soils, desert soils,

forest soils and rocks), freshwater (rivers, lakes, streams, ponds) and marine habitats (Tandeau de Marsac and Houmard, 1993). As extremotrophs or extremophiles, many cyanobacteria grow and thrive in extreme environments, such as ice-based cold habitats (Mueller *et al.,* 2005; Pandey *et al.,* 1995), geothermal habitats (Ward and Castenholz, 2000), hypersaline habitats (Oren, 2000) and deserts (Wynn-Williams, 2000). They possess effective protective and tolerance mechanisms against various abiotic stresses, such as desiccation (Potts, 1999), salinity (Hagemann, 2011), ultraviolet radiation (Ehling-Schulz and Scherer, 1999; Quesada and Vincent, 1997), high light intensity (Donkor and Häder, 1995; Lakatos *et al.*, 2001), extremes of temperature (Hossain and Nakamoto, 2002; Singh *et al.,* 2005; Tang and Vincent, 1999), oxidative (Hossain and Nakamoto, 2003), acid (Gopalaswamy *et al.*, 2007) and heavy metals (Turner and Robinson, 1995).

Moreover, they have evolved efficient mechanisms to cope with nutrient-limiting conditions in ambient environment. For instance, to cope with phosphate-limiting conditions, they store intracellular polyphosphate reserves which allow them to withstand short periods of phosphate deprivation, and produce extracellular phosphatases to obtain phosphate from organic substrates present in the surrounding medium (Carr and Mann, 1994; Grossman *et al.*, 1994). Similarly, depletion of combined nitrogen sources leads to expression of nitrogenase activity in diazotrophic (nitrogen-fixing) cyanobacteria which allow them to use molecular dinitrogen as nitrogen sources. The ability of many cyanobacteria to perform both photosynthesis and nitrogen fixation together with their efficient nutrient uptake mechanisms and adaptation to low light intensity makes them highly productive and efficient biological system.

Occurrence and Biological Roles of Phosphorus

Phosphorus (symbol, P; atomic number, 15) is a biologically important element that is essential for all life forms-microbes, plants, animals and humans. Chemically, it is a multivalent non-metal of the nitrogen group. Elemental phosphorus exists in two major forms- white phosphorus and red phosphorus, and is highly toxic. But due to its high chemical reactivity, phosphorus is never found as free element (pure form) on Earth. It occurs mainly as phosphate (PO_4^{3-}) or as organophosphate. It is widely distributed in different minerals. Microorganisms are intimately involved in the cycling of phosphorus in terrestrial and aquatic ecosystems. They participate in the solubilization of inorganic phosphorus and in the mineralization of organic phosphorus (organically bound phosphorus). They are important in immobilization of available phosphorus. Phosphorus exists in nature in a variety of inorganic and organic forms, but primarily in either insoluble or only poorly soluble inorganic forms. By far the largest reservoir of phosphorus is oceanic sediments followed by soil, dissolved (inorganic) form in ocean, mineral rock, land biota, detritus (particulates) in ocean, ocean biota and freshwater (dissolved) in descending order (Borie and Zuniono, 1983). Except for very limited microbial transformations, phosphorus exists in the environment as orthophosphate. In this form phosphorus is readily taken up by both aquatic and terrestrial plants and microbes. Orthophosphate ion (PO_4^{3-}), usually and simply called phosphate, is a polyatomic ion, consisting of one central phosphorus atom surrounded by four oxygen atoms in a tetrahedral arrangement. In biological systems, phosphorus is found as a free phosphate ion in solution and is called inorganic phosphate, to distinguish it from phosphate bound to various phosphate esters. Inorganic phosphate is generally denoted by P_i and at physiological (neutral) pH primarily consists of a mixture of HPO_4^{2-} and H_2PO^{4-}.

In biological systems, phosphorus has diverse structural, functional and regulatory roles. It is constituent of various biomolecules, such as nucleic acids (DNA and RNA), phospholipids (present in cell membranes), energy molecules (ATP and ADP), signaling molecules or secondary messengers (cAMP, cGMP, inositol-1,4,5-triphosphate) and sugar-phosphate (which plays role in photosynthesis and intermediary metabolism). It plays roles in various biological processes, including energy transformation, nucleic acid synthesis, glycolysis, respiration, membrane synthesis and stability, enzyme activation/inactivation, redox reaction, signaling, carbohydrate metabolism and nitrogen fixation (Vance *et al.,* 2003). It plays regulatory role in molecular, cellular, organismic and ecosystem processes (Comerford, 1998). It plays key role in regulating the primary productivity of aquatic ecosystems (Wu *et al.,* 2000). It is a key determinant of the trophic status of the water body. Phosphorus is the dominant element controlling carbon and nitrogen immobilization (Paul and Clark, 1988). Among various physical and chemical factors, the availability of phosphorus (P) greatly influence the growth, development and population dynamics of phytoplanktons, including cyanobacteria, in freshwater environments (Oliver and Ganf, 2000; Sigee, 2005). An excess input of phosphate by municipal, agricultural and industrial effluents is an important factor in eutrophication (nutrient enrichment) of freshwater ecosystems. Phosphorus bioavailability is controlled by pH, ionic strength, concentration of phosphorus and metals (Fe, Al and Ca) and the presence of competing anions, including organic acids (Sanyal and De Datta, 1991).

Phosphorus occurs in freshwater bodies as soluble organic (dissolved organic matter, DOM), insoluble organic (biota and detritus) and soluble inorganic (phosphate) forms. In most freshwater bodies, phosphorus occurs mainly as insoluble organic P. Like other phytoplanktons, assimilation of phosphorus by cyanobacteria is restricted to uptake of phosphate ions (PO_4^{3-}), which constitute the biologically available phosphorus, the concentration of which is referred to as 'total soluble phosphate' or TSP. Dissolved organic P, other soluble components, is readily converted to phosphate by phosphatase enzymes, and together with TSP, constitutes the 'biologically available phosphorus' (BAP) within the water (Sigee, 2005).

Cyanobacterial Adaptation Strategies to Phosphorus Deficiency

In order to ensure growth, development and survival under Pi-deficient conditions, cyanobacteria and other phytoplanktons have developed several strategies to cope with Pi-depletion (Falkner *et al.*, 1998; Dignum *et al.*, 2005). Firstly, the cells must possess high-affinity phosphate uptake systems that operate efficiently at very low and fluctuating Pi concentrations. This is known as affinity strategy that involves induced synthesis of high-affinity uptake system for Pi. Secondly, Pi must be stored inside the cells to secure the availability in times when Pi-uptake ceases. This is called storage strategy. This implies synthesis of enzymes that transform Pi into polyphosphate inside the cell. Polyphosphate, which acts as source of Pi, enables the cells to produce several generations when external nutrient concentrations are low. Thirdly, induced synthesis of enzymes (e.g. alkaline phosphatase) that transform generally inaccessible phosphate-containing compounds in to an utilizable form. This is called scavenging strategy.

Alkaline Phosphatase Activity in Cyanobacteria

Phosphatases are enzymes that catalyze the hydrolysis of organic phosphate esters to produce inorganic phosphate (Pi). These are ubiquitous in nature. Organisms ranging from bacteria to higher plants, including algae and cyanobacteria, produce phosphatase in response to low availability of free Pi or under the condition of phosphorus deficiency in environments (Tadano *et al.*, 1993; Jansson *et al.*, 1988; Vance *et al.*, 2003). During long periods of phosphorus deprivation or limitation cyanobacteria excrete extracellular phosphatases (APase) which catalyze the degradation of various complex organic phosphate substrates into organic moiety and biologically available inorganic phosphate (Pi), and thus is believed to have an essential function in the nutrient dynamics of most of the ecological niches (Stihl *et al.*, 2001). Phosphatases have been implicated in species competition within natural phytoplankton community (Strojsova *et al.*, 2008). On the basis of location of their activity, they are known as cell-bound or surface phosphatase and extracellular phosphatase. The term cell-bound or surface phosphatase is used when its activity is detected in centrifuged cells (Flynn *et al.*, 1986) or its activity is localized outside the cytoplasmic membrane (Whitton *et al.*, 1991). The term extracellular phosphatase is used when its activity is detected in cell-free medium or supernatant. Microorganisms with phosphatase activity are able to hydrolyze phosphate from a variety of organic phosphorus compounds. Phosphatases can be classified according to the substrates on which it acts, such as phosphomonoesterase (PMEase) which catalyses the enzymatic breakdown of monoester bonds (in phosphomonoesters) and phosphodiesterase (PDEase) which acts on diester bonds (in phosphodiesters) and so on. According to pH at which phosphatases exhibit maximum activity, they are classified as alkaline phosphatase (pH 7.6-10.0) and acid phosphatase (pH 2.6-6.8). Both alkaline and acid phosphatases have been found as external and internal enzymes in algae and bacteria but they produce more alkaline than acid phosphatases with external function (Siuda, 1984; Schmitter and Jurkiewicz, 1981; Kuenzler and Perras, 1965; Aaronson and Patni, 1976; Cembella *et al.*, 1984). In many respects, alkaline and acid phosphatases share essential characteristics and shows specificity against different substrates, i.e. their activity is restricted only to the P-O bond on the phosphoesters (Feder, 1973). However, differences have also been reported. Alkaline phosphatases often require divalent metal ions for their activity and are inhibited by chelators such as EDTA, while acid phosphatases are frequently specifically inhibited by fluoride (Cembella *et al.*, 1984). In contrast to alkaline phosphatase, the synthesis of acid phosphatase is generally not repressed by orthophosphate (Wynne, 1977). Acid phosphatases are constitutive enzymes produced mainly to serve the internal phosphorus metabolism while alkaline phosphatases have external functions and the synthesis of which depend on the ambient phosphorus level. They also show essential difference concerning their location in the cell and the mode of their synthesis.

Alkaline phosphatase (EC 3.1.3.1) is an homodimeric metaloenzyme, containing two zinc ions (Zn^{2+}) and one magnesium ion (Mg^{2+}) in each active site (one per monomer) (Bradshaw *et al.*, 1981; Bortolato *et al.*, 1999). It is a dimeric protein (constituted of two subunits) of molecular weight 160,000 daltons (Chróst, 1991). Zn^{2+} and Mg^{2+} ions are held by imidazole and carboxylate groups at the active site of the enzyme (Metzler, 2001). The active site of the enzyme is located in a pocket created by the termination of a number of helices and sheets that is open to the surface (Kim and Wyckoff, 1991). The two zinc ions are directly involved in catalysis, whereas the magnesium ion does not directly participate in the catalysis but is important for the structural stabilization of the enzyme (Simpson *et al.*, 1968; Anderson *et al.*, 1975). The two zinc ions are well positioned to activate the serine and water for nucleophilic attacks and they are involved in holding of the phosphate portion of substrate (Kim and Wyckoff, 1991). One zinc ion is required for catalysis and plays an important role in binding both the substrate and phosphate. The second one interacts with the hydroxyl group of the active serine to stabilize the deprotonated form of serine necessary for the nucleophilic attack on the phosphate. The main amino acid residues that serve as ligands to divalent ions are Asp, His, Thr and Glu (Sowadski *et al.*, 1985; Kim and Wyckoff, 1991). The alkaline phosphatases are found in bacteria, cyanobacteria, algae, fungi and higher animals, including humans, but not in higher plants. In cyanobacteria, the activity of alkaline phosphatase constitutes one of the most survival strategies to grow and survive under conditions of phosphate deficiency (Bhaya *et al.*, 2000). Its activity serves as a simple and effective indicator for phosphorus status of phytoplanktons. The extracellular APase remains active in dissolved state in natural waters where it is believed to play role in nutrient dynamics (Berman, 1969; Kobayashi *et al*., 1984). Secretion of the enzyme is induced by low, and repressed by high aquatic phosphate levels. It is also induced under conditions of internal phosphorus deficiency, when the cell quota falls to critically low levels. Hydrolysis of organic phosphorus compounds by alkaline phosphatases leads to increased phosphorus uptake of algal or cyanobacterial cells and increased rates of nutrient cycling. The ability of cyanobacterial cells to release phosphate ions from soluble organic phosphates in the aquatic environment is particularly important under P-limiting conditions, where differences between species in terms of induction and amount of enzyme produced may be an important factor in inter-specific competition. Most of the alkaline phosphatases are located in the periplasmic space (the space between the cytoplasmic membrane and the outer membrane) of cell wall of bacteria and cyanobacteria as soluble enzymes (Cheng and Costerton, 1973; Bhatti *et al.*, 1976; McNicholas and Hulett, 1977; Malamy and Horecker, 1961; Thompson and McLeod, 1974; Doonan and Jensen, 1977) but membrane-associated enzymes have also been found (Von Tigerstrom and Stelmaschuk, 1986; Baoudene-Assali *et al.*, 1993). The most thoroughly investigated prokaryotic alkaline phosphatase is that of *Escherichia coli* (Wanner, 1987).

Alkaline phosphatase activity primarily depends on the type and concentration of the substrate and the enzyme. Other factors affecting its activity are light intensity, temperature, ionic strength and metal ions (McComb *et al.*, 1979). The cellular phosphate pool has been reported to regulate the synthesis or activity of alkaline phosphatase in cyanobacteria. The activity of alkaline phosphatase is inversely proportional to the decrease or increase in cellular phosphate levels (Fitzerald and Nelson, 1966; Kumar *et al.*, 1992). Low phosphatase activity is assumed to be a consequence of the repression of phosphatase synthesis caused by high cellular P content (Chróst,1991) and high cellular P content leads (if microbial growth is P-limited) to a high growth rate (Droop,1973). Conversely, high phosphatase activity should occur at low cellular P content, which also implies a low growth rate (Vadstein *et al*., 1988).

Alkaline phosphatases have certain potential applications or practical use. Measurement of phosphatase activity of organisms is recognized as an important practical biochemical tool in limnological and sonographic studies. In conjugation with other indicators, it can help to assess the degree of P limitation in an aquatic ecosystems or a single species. Alkaline phosphatase assay can be used for monitoring chemical pollution in freshwater ecosystems (Durrieu *et al.,* 2003). Phosphatase activity can be used as an early warning indicator of wetland eutrophication (Newman et al., 2003). Phosphatases have also been suggested to be involved in other metabolic processes, such as transport of substances across membranes and synthesis of new organic phosphates (transphosphorylation) (McComb *et al.,* 1979). The phosphatase activity also provides information about the utilization of dissolved organic phosphorus (DOP) as an additional source of phosphate by bacteria and primary producers (Hernández *et al*., 2002). Phosphatases are a significant component of most marine algae and bacteria and play a prominent role in the recycling of organic phosphorus and in the avoidance of phosphorus limitation in the sea (Hoppe, 2003).

Conclusion

In freshwater ecosystems, phosphorus is often the limiting nutrient for growth of various phytoplanktons, including cyanobacteria, and its bioavailability is an important factor regulating phytoplankton productivity and species composition. Alkaline phosphatase is an important enzyme produced by phytoplanktons that hydrolyze complex organic phosphorus compounds to liberate orthophosphate, the form of phosphorus directly available to biota. Alkaline phosphatase activity is regarded as a common indicator of phosphorus deficiency or stress in phytoplanktons as well as in freshwater environments. Phosphorus is the principal nutrient responsible for the eutrophication of lakes and reservoirs globally. For the management of trophic state of lakes and reservoirs, it is important to understand the mechanism of orthophosphate regeneration by biota which mainly depends upon the productivity of extracellular alkaline phosphatase by them. Often, an inverse relationship exists between the activity of alkaline phosphatase and the concentration of orthophosphate in lake water. The activity of alkaline phosphatase in water can be used as the determinant of the trophic status of lakes and other freshwater bodies.

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REFERENCES

Aaronson S and Patni NJ (1976). The role of surface and extracellular phosphatases in the phosphorus requirement of *Ochromonas*. *Limnology and Oceanography* **21** 838-845.

Abed RMM, Dobretsov S and Sudesh K (2009). Applications of cyanobacteria in biotechnology. *Journal of Applied Microbiology* **106** 1-12.

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

Anderson R, Bosron W, Kennedy F and Vallee B (1975). Role of magnesium in Escherichia coli alkaline phosphatase. *Proceedings of National Academy of Science* **72** 2989-2993.

Baoudene-Assali F, Baratti J and Michel GPF (1993). Purification and properties of a phosphate-irrepressible membrane-bound alkaline phosphatase from *Zymomonas mobilis. Journal of General Microbiology* **139** 229-235.

Berman T (1969). Phosphate release of inorganic phosphorus in lake Kinneret. *Nature* **224** 1231-1232.

Bhatti AR, De Voe IW and Ingram JM (1976). The release and characterization of some periplasm-located enzymes of *Pseudomonas aeruginosa*. *Canadian Journal of Microbiology* **22** 1425-1429.

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

Borie F and Zuniono H (1983). Organic matter P association as a sink in P fixation process in allophonic soils in Chile. *Soil Biology and Biochemistry* **15** 599-603.

Bortolato M, Besson F and Roux B (1999). Role of metal ions on the secondary and quaternary structure of alkaline phosphatase from bovine intestinal mucosa. *Proteins: Structure, Function and Genetics* **37** 310-318.

Bradshaw RA, Cancedda F, Ericsson LH, Neumann PA, Piccoli SP, Schlesinger MJ, Shriefer K and Walsh KA (1981). Amino acid sequence of Escherichia coli alkaline phosphatase. *Proceedings of National Academy of Science* **78** 3473-3477.

Carr NG and Mann NH (1994). The oceanic cyanobacterial picoplankton. In: *The molecular biology of cyanobacteria*, ed. Bryant DA (Kluwer Academic Publishers, Dordrecht, Boston and London) 27-48.

Castenholz RW and Waterbury JB (1989). Oxygenic photosynthetic bacteria, group I cyanobacteria. In: *Bergey's manual of systematic bacteriology*, Vol-3, eds. Stanley JT, Bryant MP and Pfennig N (Williams and Wilkins, Baltimore, Maryland) 1710-1798.

Cembella AD, Antia NJ and Harrison PJ (1984). The utilization of inorganic and organic phosphorus compounds as nutrients by eukaryotic microalgae: a multidisciplinary perspective: *Part 1. CRC Critical Reviews in Microbiology* **10** 317-391.

Cheng KL and Costerton JN (1973). Localization of alkaline phosphatase in three Gram-negative rumen bacteria. *Journal of Bacteriology* **116** 424-440.

Chróst RJ (1991). Environmental control of the synthesis and activity of aquatic microbial ectoenzymes. In: *Microbial enzymes in aquatic environments*, ed. Chrost RJ (Springer- Verlag, New York) 29-59.

Comerford NB (1998). Soil phosphorus bioavailability. In: *Phosphorus in plant biology: regulatory roles in molecular, cellular, organismic and ecosystem processes*, eds. Lynch JP and Deikman J (American Society of Plant Physiology, Rockville, M.D., USA) 136-147.

De Phillips R, Paperi R, Sili C and Vincenzini M (2003). Assessment of metal removal capability of two capsulated cyanobacteria, *Cyanospira capsulata* and *Nostoc* PCC7936. *Journal of Applied Phycology* **15** 155-161.

Dignum M, Matthijs HCP, Pel R, Laanbroek HJ and Mur LR (2005). Nutrient limitation of freshwater cyanobacteria. In: *Harmful cyanobacteria*, eds. Huisman J, Matthijs HCP and Visser PM (Springer, Netherlands) 65-86.

Donkor VA and Häder DP (1995). Protective strategies of several cyanobacteria against solar radiation. *Journal of Plant Physiology* **145** 750-755.

Doonan BB and Jensen TE (1977). Ultrastructural localization of alkaline phosphatase in the blue-green bacterium *Plectonema boryanum*. *Journal of Bacteriology* **132** 967-973.

Droop MR (1973). Some thoughts on nutrient limitation in algae. *Journal of Phycology* **9** 264-272.

Durrieu C, Badreddine I and Daix C (2003). A dialysis system with phytoplankton for monitoring chemical pollution in freshwater ecosystems by alkaline phosphatase assay. *Journal of Applied Phycology* **15** 289-295.

Ehling-Schulz M and Scherer S (1999). UV protection in cyanobacteria. *European Journal of Phycology* **34** 329-338.

Falkner R, Wagner F, Aiba H and Falkner G (1998). Phosphate-uptake behaviour of a mutant of *Synechococcus* sp. PCC 7942 lacking one protein of the high-affinity phosphate-uptake system. *Planta* **206** 461-465.

Feder J (1973). The phosphatases. In: *Environmental phosphorus handbook*, eds. Griffith EJ, Beeton A, Spencer JM and Mitchell DT (J. Wiley & Sons, NY) 475-508.

Fischer WW (2008). Life before the rise of oxygen. *Nature* **455** 1051-1052.

Fitzgerald GP and Nelson C (1966). Extractive and enzymatic analysis for limiting or surplus phosphorus in algae. *Journal of Phycology* **2** 32-37.

Flynn KJ, Opik H and Syrett PJ (1986). Localization of alkaline phosphatase and 5′-nucleotidase activities of the diatom *Phaeodactylum tricornutum*. *Journal of General Microbiology* **132** 289-298.

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

Gopalaswamy G, Karthikeyan, Raghu R, Udayasuriyan V and Apte SK (2007). Identification of acid-stress-tolerant proteins from promising cyanobacterial isolates. *Journal of Applied Phycology* **19** 631-639.
Grossman AR, Schaefer MR, Chang GG and Collier JL (1994). The responses of cyanobacteria to environmental conditions: light and nutrients. In: *The molecular biology of cyanobacteria*, ed. Bryant DA (Kluwer Academic Publisher, Dordrecht, Boston and London) 641-675.
Hagemann M (2011). Molecular biology of cyanobacterial salt acclimation. *FEMS Microbiology Review* **35** 87-123.
Hernández I, Niell, FX and Whitton BA (2002). Phosphatase activity of benthic marine algae. An overview. *Journal of Applied Phycology* **14** 475-487.
Hoppe HG (2003). Phosphatase activity in the sea. *Hydrobiologia* **493** 187-200.
Hossain MM and Nakamoto H (2002). HtpG plays a role in cold acclimation in cyanobacteria. *Current Microbiology* **44** 291-296.
Hossain MM and Nakamoto H (2003). Role for the cyanobacterial HtpG in protection from oxidative stress. *Current Microbiology* **46** 70-76.
Jansson M, Olsson H and Pettersson K (1988). Phosphatase: origin, characteristics and function in lakes. *Hydrobiologia* **170** 157-175.
Kim EE and Wyckoff HW (1991). Reaction mechanism of alkaline phosphatase based on crystal structures. *Journal of Molecular Biology* **218** 449-464.
Kobayashi K, Hashimoto S, Fuziwara K, Haraguchi H, Fuwa K and Otsuki A (1984). Correlation between dissolved alkaline phosphatase and orthophosphate in lake water. *Chemistry Letters* **13(4)** 565-568.
Kuenzler EJ and Perras JP (1965). Phosphatases of marine algae. *Biolgical Bulletin* **128** 271-284.
Kumar TA, Singh S and Tiwari DN (1992). Alkaline phosphatase activities of an *Anabaena* from deep-water rice. *World Journal of Microbiology and Biotechnology* **8** 585-588.
Lakatos M, Bilger W and Büdel B (2001). Carotenoid composition of terrestrial cyanobacteria: response to natural light conditions in open rock habitats in Venezuela. *European Journal of Phycology* **36** 367-375.
Malamy M and Horecker BL (1961). The localization of alkaline phosphatase in *E.coli* K_{12}. *Biochemical Biophysical Research Communication* **5** 104-108.
Margulis L (1975). The microbes' contribution to evolution. *Biosystems* **7** 266-292.
Mccomb RB, Bowers, GN and Posen S (1979). Alkaline Phosphatases. Plenum press, New York.
McNicholas JM and Hulett FM (1977). Electron microscope histochemical localization of alkaline phosphatase(s) in *Bacillus licheniformis*. *Journal of Bacteriology* **129** 501-515.
Metzler DE (2001). Biochemistry: the chemical reactions of living cells, Vol.I Harcourt Academic Press, London 588-675.
Mueller DR, Vincent WF, Bonilla S and Laurion I (2005). Extremotrophs extremophiles and broadban pigmentation strategies in high arctic ice shelf ecosystem. *FEMS Microbiology Ecology* **53** 73-87.
Newman S, Mccormick PV and Backus JG (2003). Phosphorus activity as an early warning indicator of wetland eutrophication: problems and prospects. *Journal of Applied Phycology* **15** 45-59.
Oliver RL, and Ganf GG (2000). Freshwater Blooms. In: *The ecology of cyanobacteria: their diversity in time and space*, eds.Whitton BA and Potts M (Kluwer Academic Publishers, Dordrecht, the Netherlands) 149-194.
Oren A (2000). Salts and Brines. In: *The ecology of cyanobacteria: their diversity in time and space*, eds.Whitton BA and Potts M (Kluwer Academic Publishers, Dordrecht, the Netherlands) 281-306.
Pandey KD, Kashyap AK and Gupta RK (1995). Nutrient status, algal and cyanobacterial flora of six streams of Schirmacher Oasis, Antarctica. *Hydrobiologia* **299** 83-91.
Pandey VD (2010). Bioremediation of heavy metals by microalgae. In: *Algal biotechnology*, ed. Das MK (Daya Publishing House, Delhi) 287-296.
Patterson GML (1996). Biotechnological applications of cyanobacteria. *Journal of Scientific & Industrial Research* **55** 669-684.

Paul EA and Clark FE (1988). Soil microbiology and biochemistry. Academic Press, New York 222-232.
Potts M (1999). Mechanisms of dessication tolerance in cyanobacteria. *European Journal of Phycology* **34** 319-328.
Quesada A and Vincent WF (1997). Strategies of adaptation by Antarctic cyanobacteria to ultraviolet radiation. *European Journal of Phycology* **32** 335-342.
Rai AN, Soderback E and Bergman B (2000). Cyanobacteria-plant symbioses. *New Phytologist* **147** 449-481.
Sanyal SK, and De Datta SK (1991). Chemistry of phosphorus transformations in soil. *Advances in Soil Science* **16** 1-20.
Schmitter RE and Jurkiewicz AJ (1981). Acid phosphatase localization in periodic-acid Schiff bodies of *Gonyaulax*. *Journal of Cell Science* **51** 15-24.
Sigee DC (2005). Freshwater microbiology: biodiversity and dynamic interactions of microorganisms in the aquatic environment. John Wiley & Sons, Ltd., Hoboken, USA 235-286.
Simpson RT, Vallee, BL and Tait GH (1968). Alkaline phosphatase of *Escherichia coli*: composition. *Biochemistry* **7** 4336-4342.
Singh AP, Asthana RK, Kayastha AM and Singh SP (2005). A comparision of proline, thiol levels and GAPDH activity in cyanobacteria of different origins facing temperature stress. *World Journal of Microbiology and Biotechnology* **21** 1-9.
Siuda W (1984). Phosphatases and their role in organic phosphorus transformation in natural waters. A review. *Polish Archives of Hydrobiology* **31** 207-233.
Sowadski JM, Handschumacher MD, Murthy HMK, Foster B and Wickoff HW (1985). Refined structure of alkaline phosphatase from Escherichia coli at 2.8Å resolution. *Journal of Molecular Biology* **186** 417-433.
Stal LJ (1995). Physiological ecology of cyanobacteria in microbial mats and other communities. *New Phytologist* **131** 1-32.
Stanier RY and Cohen-Bazire G (1977). Phototrophic prokaryotes: The cyanobacteria. *Annual Review of Microbiology* **31** 225-274.
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. *Journal of Phycology* **37** 310-317.
Strojsova A, Nedoma J, Strojsova M, Cao X and Vrba J (2008). The role of cell-surface-bound-phosphatases in species competition within natural phytoplankton assemblage: an *in situ* experiment. *Journal of Limnology* **67** 128-138.
Tadano T, Ozawa K, Saki H, Osaki M and Matsui H (1993). Secretion of acid phosphatase by the roots of crop plants under phosphorus-deficients and some properties of the enzyme secreted by lupin roots. *Plant Soil* **155/156** 95-98.
Tandeau de Marsac N and Houmard J (1993). Adaptation of cyanobacteria to environmental stimuli: new steps towards molecular mechanisms. *FEMS Microbiology Review* **104** 119-190.
Tang EY and Vincent WF (1999). Strategies of thermal adaptation by high latitude cyanobacteria. *New Phytologist* **142** 315-323.
Thompson LMM and Mac Leod RA (1974). Biochemical localization of alkaline phosphatase in the cell wall of a marine *Pseudomonad. Journal of Bacteriology* **117** 819-825.
Tomitani A, Knoll AH, Cavanaugh CM and Ohno T (2006). The evolutionary diversification of cyanobacteria: molecular-phylogenetic and paleontological perspectives. *Proceedings of National Academy of Science* 103 5442-5447.
Turner JS and Robinson NJ (1995). Cyanobacterial metallothioneins: Biochemistry and molecular genetics. *Journal of Industrial Microbiology* **14** 119-125.
Vadstein O, Jensen A, Olsen Y and Reinertsen H (1988). Growth and phosphorus status of limnetic phytoplankton and bacteria. *Limnology and Oceanography* **33** 489-503.
Vance CP, Uhde-stone C and Allan DL (2003). Phosphorus acquisition and use: critical adaptations by plants for securing a nonrenewable resource. *New Phytologist* **157** 423-447.

Von Tigerstrom RG and Stelmaschuk S (1986). Purification and characterization of the outer membrane associated alkaline phosphatase of *Lysobacter enzymogens. Journal of General Microbiology* **132** 1379-1387.

Wanner BL (1987). Bacterial alkaline phosphatase gene regulation and the phosphate response in *Escherichia coli.* In: *Phosphate metabolism and cellular regulation in microorganisms*, eds. Torriani-Gorini A, Rothman FG, Silver S, Wright A and Yagil E (American Society of Microbiology, Washington DC)127-157.

Ward DM and Castenholz RW (2000). Cyanobacteria in geothermal habitats. In: *The ecology of cyanobacteria: their diversity in time and space*, eds.Whitton BA and Potts M (Kluwer Academic Publishers, Dordrecht, the Netherlands) 37-59.

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

Whitton BA (1973). Interaction with other organisms. *In*: The biology of blue-green algae, eds. Carr NG and Whitton BA (Blackwell Scientific Publications, Oxford) 415-432.

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

Wu J, Sunda W, Boyle EA and Karl DM (2000). Phosphate depletion in the western North Atlantic ocean. *Science* **289** 759-762.

Wynne D (1977). Alterations in activity of phosphatases during the *Peridinium* bloom in Lake Kinneret. *Physiologia Plantarum* **40** 219-224.

Wynn-Williams DD (2000). Cyanobacteria in deserts-life at the limit. In: *The ecology of cyanobacteria: their diversity in time and space*, eds.Whitton BA and Potts M (Kluwer Academic Publishers, Dordrecht, the Netherlands) 341-366.