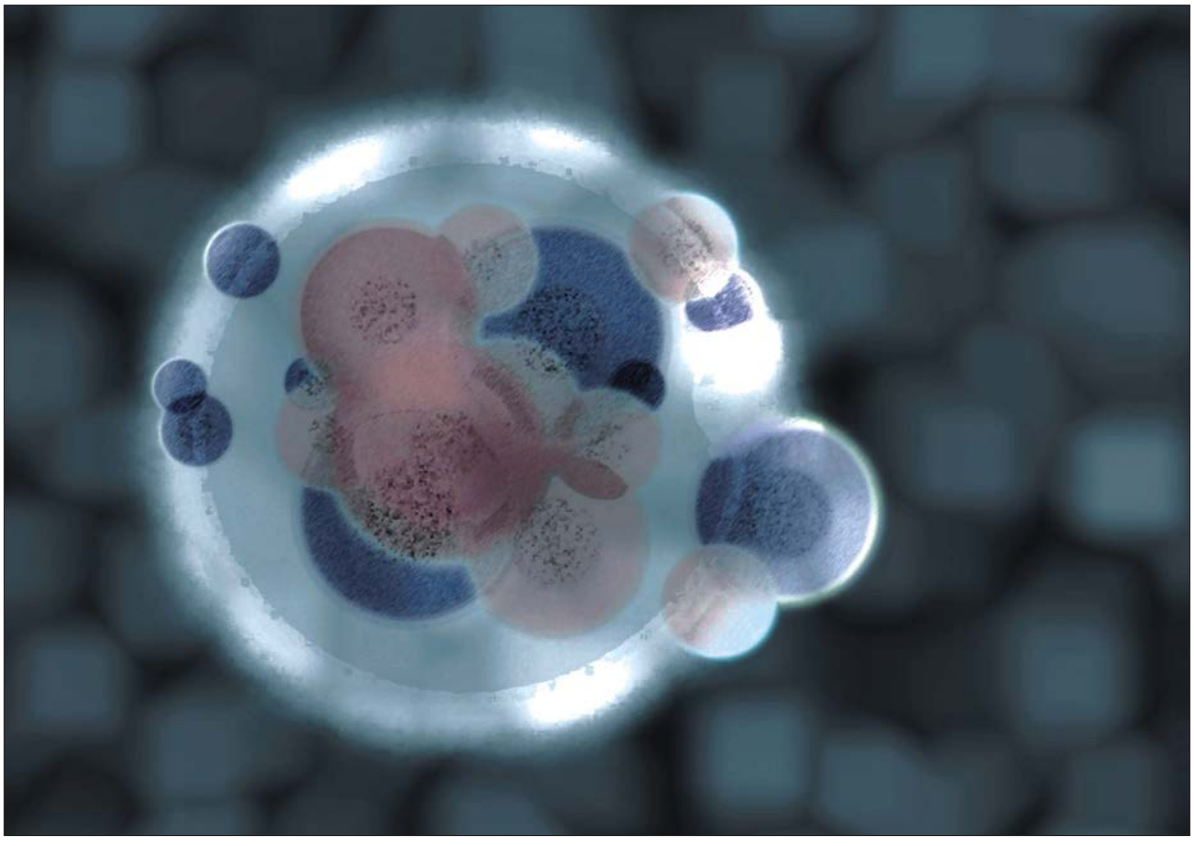

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Factors Affecting Alkaline Phosphatase Activity of the Marine Cyanobacterium *Lyngbya majuscula*

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Abstract: Some environmental factors affecting phosphatase activity of the cyanobacterium *Lyngbya majuscula* found on the coral reefs in the Red Sea were investigated. Phosphatase activity of *L. majuscula was* restricted only to phosphomonoesterase (PMEase) without any detectable level of phosphodiesterase (PDEase) during all experiments. The maximum enzyme activity was obtained at 35°C (69.1 *p*NP μmol mg^{-1} dry wt. h^{-1}) and at pH 10 (89.1 mol *p*NP mg^{-1} dry wt. h^{-1}). The activity was markedly inhibited by Fe, Zn, Na, K and P ions at high concentrations (1 and 10 mM), but lower concentrations (0.01 and 0.1 mM) enhanced this activity. The highest concentration of Ca and Mg ions (10 mM) inhibited the enzyme activity, while lower concentrations stimulated this activity. Salinity had a marked effect on the enzyme activity, with a maximum obtained at 5‰.

Key words: *Lyngbya majuscula*, phosphatase, activity, Red Sea

INTRODUCTION

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). Although nitrogen, rather than, phosphorus, is the growth limiting factor for phototrophic organisms in sea (Tyrrell, 1999), phosphorus has been recorded as limiting factor, at least for certain species in costal and offshore regions (Litter *et al*., 1991; Lapointe *et al*., 1992, Thingstand and Rassoulzadegan, 1995; Cotner *et al*., 1997; Rivkin and Anderson, 1997; Zohary and Robarts, 1998). Phosphorus supply in the sea depends largely on the regeneration of phosphate from dissolved organic matter (Harrison, 1983). The regeneration process is carried out by production of exoenzymes such as 5-nucleotidase and phosphatase by phytoplankton (Ammerman and Azam, 1991).

Cyanobacteria contribute significantly to phytoplankton communities in marine oligotrophic waters (Stihl *et al*., 2001). The ability of cyanobacteria to adapt to low inorganic phosphorus environments through hydrolysis of organic phosphate by phosphatases has been studied (Grainger *et al*., 1989; Mahasneh *et al*., 1990; Whitton *et al*., 1990, 1991, 1998; Palenik and Dyhrman, 1998; Scanlan and Wilson, 1999; Stihl *et al*., 2001). Adaptation of cyanobacteria to low ambient phosphate concentrations involves the expression of the *PstS* gene, encoding a perisplasmic phosphate-binding protein involved in transport (Scanlan *et al*., 1993) and *phoA* gene, encoding Alkaline phosphatase (AP), a monophosphate-esterase that separates phosphate from a wide spectrum of organic P compounds.

From our viewpoints, the most important bloom-forming filamentous cyanobacterial species in the marine environments are *Trichodesmium* and *Lyngbya majuscula*. The latter has attracted interest to study for three reasons. Firstly, *L. majuscula* has been recently recorded as one of cyanobacterial species having particular physiological strategies that permit them to fix nitrogen under well-oxygenated conditions even without a heterocyst (Whitton and Potts 2000; Burja *et al*., 2002). Secondly, the organism is known to produce deramtotoxins called lyngbyatoxin A, which is responsible for a severe erythromatous, papulavesicular dermatitis known as swimmers itch (Aimi *et al*., 1990). The third reason is that *L. majuscula* has been proven to be a prolific source of novel secondary metabolites of interest to pharmaceutical companies (Burja *et al*., 2002; Mohamed *et al*., 2002).

Increased dominance of benthic cyanobacteria and macroalgae on coral reefs is a continuing phenomenon in near shore tropical environments, particularly in areas impacted by humans (Hughes *et al*., 1999; McCook, 1999). Of particular, *Lyngbya majuscula* which was frequently observed to form mats on the surface of coral reefs at Farasan Island in the Red Sea, Saudi Arabia, where soluble phosphate is very low. Epidemiological studies demonstrated the implication of this species in deramtotoxic diseases for humans swimming in Farasan

island water contaminated with *L. majuscula*. Laboratory studies showed that *L. majuscula* isolated from Farsan island exhibited dermatotoxic effects to mice (Al-Shehri and Mohamed, 2006). Therefore, the present study was undertaken to study some environmental factors affecting the phosphatase activity of such a toxic cyanobacterium in order to control its growth and distribution in Saudi Arabian waters.

MATERIALS AND METHODS

Organism: *Lyngbya majuscula* tufts were collected by hand from the surface of coral reefs in the Farasan Island. This island is subjected to human activities and thus increasing the content of organic compounds in this area. The Farasan Island is 3310 km^2 and is located in the southern Red Sea within the borders of Saudi Arabia, 42 km offshore of the coastal city of Jazan (16°40'N and 42° 00'E). The temperature in this area does not decrease below 30°C allover the year. *L. majuscula* tufts were transported in a container with seawater to the laboratory within 6 h. Set-up of the experiments was carried out within 12 h. The filaments of *L. majuscula* were washed several times with sterilized seawater, homogenized under sterilized conditions and streaked on agar plates containing k medium. After staining, the cultures was investigated under microscope to ensure the absence of any attached bacteria. The organism was identified as *Lyngbya majuscula* according to John *et al.* (2002). *L. majuscula* was cultured in P-depleted BG11 medium to test its ability to grow under P-limited conditions.

Phosphatase activity experiments: Except the experiment of influence of salinity (NaCl) on enzyme activity, which has been carried in batch culture of *L. majuscula* using BG-11 medium, all assays of phosphatase activity were performed on aliquots of *L. majuscula* homogenate. Homogenization was carried out using a series of sterile syringes with needles and sonication at 4°C. Standard assays were carried out according to Mahasneh *et al.* (1990) with some modifications. The assays were carried out at pH 10 in medium buffered with glycine (50 mM) and NaOH and 30°C for 30 min. *p*-nitrophenylphosphate (*p*NPP) was used as enzyme substrate at a final concentration of 250 μM during all the experiments. The assay was terminated by the addition of 0.25 mL of terminating solution (27 mM of EDTA, 550 mM of K_2HPO_4 and 550 mM of NaOH). The color produced was measured at 405 nm using UV/ visible spectrophotometer (UV-1601 PC, Shimadzu Coropration, Kyoto, Japan). Activity is reported as μmol *p*NP mg^{-1} dry wt. h^{-1}, formed by the hydrolysis of *p*NPP or bis-*p*NPP to Pi and (one or two molecules, respectively) of *p*NP.

The effect of temperature on activity was tested by incubation of aliquots of tuft homogenate for 30 min. at 5 degree intervals between 20 and 60°C. the effect of pH on activity was tested using a range of buffers as used by Mahasneh *et al.* (1990). The effects of various elements on phosphomonoestearse activity were estimated using different concentrations (0, 0.01, 0.1, 1 and 10 mM) of these elements in assay mixture. The ions tested Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe-III-Chelate, Zn^{2+} and Phosphate. Cations were added as the relevant chloride or sulphate. NaOH used for buffering glycine was replaced by KOH in the case of Na assay. The influence of salinity on phosphate activity was tested by culturing *L. majuscula* in BG-11 medium supplemented with different concentration of NaCl (0, 5, 7.5 and 10‰) for 21 days at room temperature (22±2°C) under an irradiance of 40 μmol m^{-2} sec. Harvested cells were centrifuged at 10000 x g for 15 min., washed twice, re-suspended in assay medium and then homogenized by the same method mentioned above. The value of each parameter is the mean of three replicates plus standard deviation (SD) calculated using Microsoft Excel Program 2000.

RESULTS

Lyngbya majuscula grew well under P-depleted BG11 medium during the preliminary experiment testing the ability of this organism to grown under P-limited conditions (data not shown). Phosphatase activity varied with changes in physical and chemical factors. This activity of *L. majuscula* differed markedly under influence of different temperatures, with optimum at 35°C (Fig. 1). The maximum temperature at which the activity was detectable, was 60°C. The optimum pH value for the highest activity was 10 (Fig. 2).

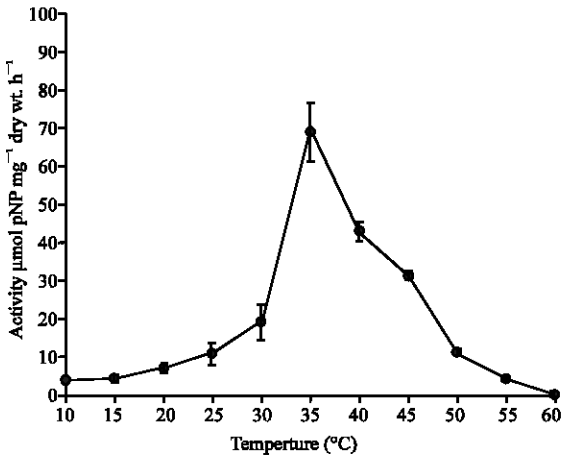

Fig. 1: Influence of temperature on phosphatase activity of *L. majuscula* at pH 10 for 30 min

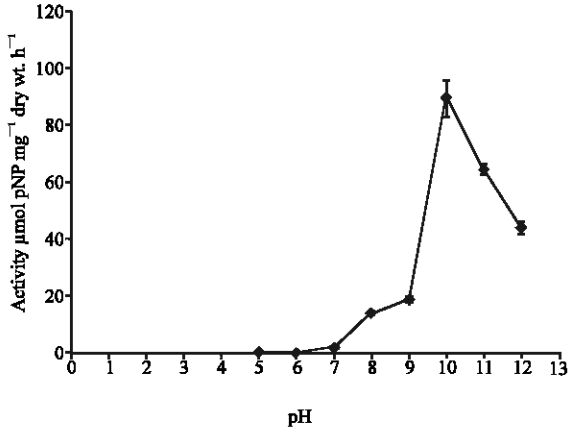

Fig. 2: Influence of pH on phosphatase activity of *L. majuscula*

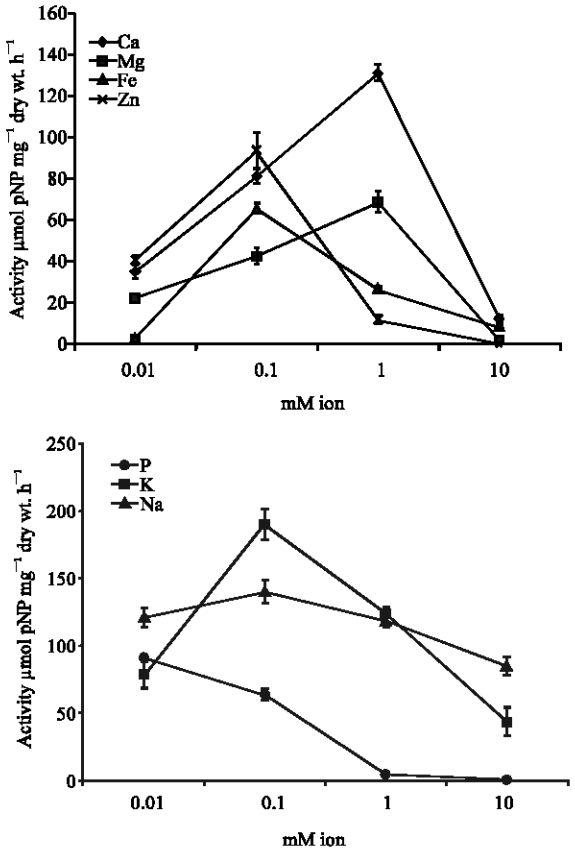

Fig. 3: Effect of ion concentrations (mM) on phosphatase activity of *L. majuscula*

Phosphatase activity was also differed markedly as influenced by ions/molecules (Fig. 3). The activity increased by elevation of Ca and Mg concentrations until reached 1 mM and then decreased sharply at 10 mM. Both Fe and Zn induced the enzyme activity

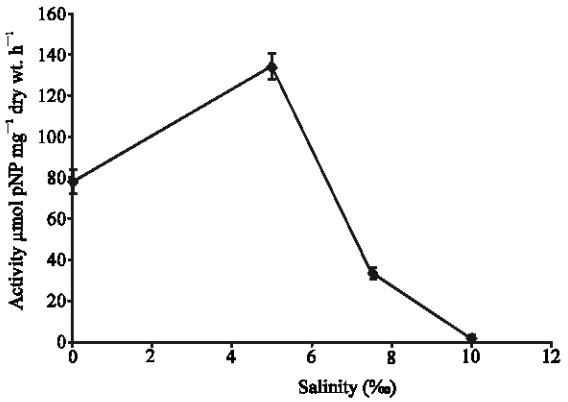

Fig. 4: Effect of salinity (‰) on phosphatase activity of *L. majuscula*

at concentrations lower than 0.1 mm, while higher levels of these ions (>1 mM) inhibited the activity. Similarly, P, Na and K ions had great inhibitory effect on the activity at concentrations more than 1 mM, but lower concentrations of these ions stimulated the activity. Salinity affected the enzyme activity, with highest rate at 5‰ salinity (Fig. 4). Moreover, the activity was much lower at high salinity levels than that obtained while no salinity in the medium.

DISCUSSION

In order to identify the possible causes of *L. majuscula* blooms in the marine environment, it is important to establish the factors that may be stimulating these blooms and ascertain what role these factors play in growth and ecology of *L. majuscula*. The availability and function of certain nutritional elements such as N and P, may hold the key to identifying the cause of such blooms and provide an understanding in how to control them. Growth of phytoplankton in an area of marked P-limitation suggesting the role of alkaline phosphatase (APase) in supplying phosphorus during phosphorus depletion (Davies and Smith, 1988). Thus, high phosphatase activity gives an indication of phosphorus limitation productivity (Hernandez *et al*., 2002; Whitton *et al*., 2005). *L. majuscula* mats used in the present study, was reported to have a high phosphatase activity (Al-Shehri and Mohamed, 2006).

The temperature optimum of phosphatase activity in *L. majuscula* (35°C) is close to the range observed for other cyanobacteria (Grainger *et al*., 1989; Whitton *et al*., 1990). In benthic algae, the optimum temperature for APA is usually higher than temperatures likely to occur in nature (Hernandez *et al*., 1996a). The optima for phosphatase activity of *Nostoc commune* UTEX 584 assayed at pH 7.6 was 32°C (Whitton *et al*., 1990). The highest PMEase activity in *L. majuscula* assayed with *p*PNPas a substrate, was detected at pH 10. These results

are in coincidence with those obtained for other cyanobacteria (Healey, 1973; Grainger *et al*., 1989). Thus the enzyme can be termed as alkaline phosphatase according to Jansson *et al*. (1988).

The ionic requirements reported for cyanobacterial phosphatases vary considerably (Grainger *et al*., 1989). In this study, greater inhibition of PMEase activity of *L. majuscula*, has occurred by phosphate, while greater stimulation was by Ca and Mg. The inhibitory effect of high phosphate concentration on phosphatase activity of *L. majuscula*, was expected and agreed with several previous studies (Hernandez *et al*., 1995). However, other studies showed that phosphatase activity in some algae and macrophytes is not suppressed at high phosphate concentration (Hernandez *et al*., 1995, 1996b). These studies suggest the existence of two different phosphatase enzymes with significant activity at alkaline pH; one adaptive, whose rate of synthesis is regulated by phosphate concentration and one constitutive, independent of the external phosphate levels (Hernandez *et al*., 2002). For calcium, there are several reports of enhanced phosphatase activity in response to increased calcium (Whitton *et al*., 2005). Phosphatase activity of *Calothrix parietina* rose when calcium in the medium was increased from 0.1 to 1 mM (Grainger *et al*., 1989). Surface phosphatase activity of *Nostoc commune* UTEX 584 increased when calcium in the assay medium was increased from 1 to 10 mM, while 10 mM magnesium was slightly inhibitory (Whitton *et al*., 1990).

The results of our study also showed that Na, K, Fe and Zn ions had stimulating effect on phosphatase activity of *L. majuscula* at moderate concentrations. These results are concomitant with those reported for *Nostc commune* UTEX 584 (Whitton *et al*., 1990); sodium and potassium had a little effect over the range 0.001-10 mM. Surface phosphatase activity of *N. commune* decrease to the half when Zn concentration increased from 0.001 to 0.1 mM. The effect of salinity on alkaline phosphatase activity (APA) seems to be attributable not only to the ionic strength, but to a specific effect of particular cations as Na^+ and Mg^{2+} (Hernandez *et al*., 2002). Here in our study, we used NaCl to study the effect of salinity on phosphatase activity of *L. majuscula*. Both low and high salinities inhibited the phosphatase activity of this cyanobacterium. Similarly, It has been shown that phosphatase activity of *Gelidium*, Rhodophyta, is very low under low salinities and increases with increasing salinity up to 45-50‰, where maximum APA was found (Hernandez *et al*., 1995). However, a laboratory culture of *Calothrix vigieri*, originally isolated from a mangrove root, showed very low APA in saline medium, but a marked increase within a day of transfer to freshwater medium (Mahsneh *et al*., 1990).

Based on the current study, alkaline phosphatase which is the driving force behind bloom formation of toxic *L. majuscula* on the surface of coral reefs, are governed by environmental factors. These factors should be considered during monitoring these toxic cyanobacteria in the marine environment. Further studies are also needed to predict the effect of mucus produced by Corals under stress infection (black band disease) caused by the cyanobacterium, *Phormidium corallyticum* (Richardson and Kuta, 2003), on phosphatase activity of *L. majuscula* and other bacteria on corals.

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