J. Microbiol. Biotechnol. (2005), **15**(1), 125–130

JOURNAL OF MICROBIOLOGY AND BIOTECHNOLOGY
The Korean Society for Microbiology and Biotechnology

Comparative Studies on Growth and Phosphatase Activity of Endolithic Cyanobacterial Isolates of *Chroococcidiopsis* from Hot and Cold Deserts

BANERJEE, MEENAKSHI* AND DEBKUMARI SHARMA

Laboratory of Algal Biotechnology, Department of Bioscience, Barkatullah University, Bhopal, 462026, India

Received: March 29, 2004
Accepted: September 20, 2004

Abstract The growth and phosphatase (phosphomonoesterase) activity of *Chroococcidiopsis* culture isolated from the cryptoendoliths of the Antarctic were compared with a similar isolate from the Arizona hot desert. Such cyanobacteria living inside rocks share several features with the immobilized cells produced in the laboratory. This study has relevance because the availability of phosphorus is a key factor influencing the growth of these cyanobacteria in nature, in such unique ecological niches as the hot and cold deserts. Phosphatase activity therefore is of particular importance for these organisms if they are to survive without any other source of phosphorus availability. Also, there is paucity of knowledge regarding this aspect of study in cyanobacterial cultures from these extreme environments. The salient feature of this study shows the importance of specific pH and temperatures for growth and phosphatase activity of both cultures, although there were marked differences between the two isolates. The pH and temperature optima for growth and phosphatase activity (PMEase) of *Chroococcidiopsis* 1 and 2 were 9.5, 20°C and 8.5, 40°C respectively. The K_m and V_{max} values of cultured *Chroococcidiopsis* 1 showed lower affinity of PMEase for the substrate compared to the enzyme affinity of the same organism when found within the rocks; *Chroococcidiopsis* 2 and Arizona rocks containing the same alga however showed similar affinity of PMEase for the substrate. An interesting observation was the similarity in response of immobilized *Chroococcidiopsis* 1 culture and the same organism in the Antarctic rocks to low light and low temperature stimulation of PMEase. This thermal response seems to be related to the ability of the immobilized Antarctic isolate and the rocks to either cryoprotect the PMEase or undergo a change to save the enzyme from becoming nonfunctional under low temperatures. The free cells of *Chroococcidiopsis* 1 culture however did not show such responses.

Key words: *Chroococcidiopsis*, endolithic, cyanobacteria, phosphomonoesterase, immobilization, whole communities, Antarctic, Arizona

*Corresponding author
E-mail: meenakshi_banerjee@indiatimes.com

The Antarctic is a remarkable continent: remote, hostile, and uninhabited. It is a place of extremes with the highest, coldest, and windiest environments and with very little snowfall; most of the continent is technically a desert [27, 31, 20]. Areas of extensive rock exposure where even the glaciers fail to invade are known as the Ross Desert of the McMurdo Dry Valley and represent one of the harshest environments of our planet. Examples of other such extreme habitats are the hot deserts like that in Arizona USA. The surface of these hot and cold deserts, with the exception of small protected niches such as cracks and depressions in rocks, is practically abiotic. Communities of indigenous life forms are in fact hidden. The cryptoendolithic communities exist under the surface of the rocks colonized by microorganisms, which occupy a sharply defined zone under the thin rock crust. The dominant organisms in these habitats are either the cyanobacteria, or infrequently, fungi and algae forming lichen associations [22, 25, 14, 30]. Among the cyanobacterial species, the most dominant form is the unicellular cyanobacterium *Chroococcidiopsis*, which is the most primitive organism of this group and is found to grow where all other organisms fail to grow [3, 11, 12, 13].

Microbial life in the deserts is poised at the limits of survival. Water is the primary limiting factor in both hot and cold deserts and melting snow is the only source of water in the Antarctic. There are many other physical, chemical, and ecological factors, which are influential in maintaining the delicate balance swinging from survival to their establishment or extinction. They survive because they are able to interact within their niche and either exploit its attributes or modify it to make it more suitable [2, 3, 5, 30, 31, 14, 22, 8].

Most studies on nutrient uptake and cycling in endolithic communities of hot and cold deserts have dealt with carbon [4, 21] and there are no reports on phosphorus and nitrogen metabolism. Results on whole communities (rock samples) of the Antarctic [2, 3] showed for the first time low temperature and low light adaptations for the phosphatase

enzyme and high rate of P uptake by *Chroococcidiopsis*, the dominant flora within these rocks. There are however no reports on phosphatase activity of cyanobacterial isolate from endoliths of either the Antarctic or any hot desert. There is almost complete lack of knowledge regarding the ecology, survival mechanisms, physiology, metabolic pathways, and applications of these permanently immobilized cells within the rocks.

Such microorganisms living inside the rocks share several features with the immobilized cells in those laboratory systems where limited metabolic activity is required. For instance, their location is fixed in relation to substratum, and in most circumstances, it would seem advantageous for the organism to grow only very slowly. Laboratory studies on immobilized cyanobacteria and eukaryotic algae have been carried out with several aims, including long-term preservations [24], removal of materials from the environment [29], production of specific extracellular products [10], and the development of biological systems as sensitive as environmental probes [1]. In all, some metabolic activity is required at low levels. A study of naturally immobilized cells might provide information useful to the development of such laboratory-immobilized systems. We therefore decided to compare the growth and phosphatase activity of *Chroococcidiopsis* isolates in culture from the hot and cold deserts of Antarctic and Arizona and compare the properties with the same cultured organisms in immobilized conditions in the laboratory as well as the organisms immobilized in its natural environment within the rocks for a comparison.

MATERIALS AND METHOD

Test Organism and Sampling Sites

Axenic cultures of the endolithic cyanobacterium Chroococcidiopsis, the only phototroph present in the rocks of both Antarctic (from Barwick boulder) and Arizona, U.S.A., were isolated from the rocks (Courtesy late Dr. D. D. Wynn Williams and Charles Cockles of British Antarctic Survey and Dr. Bukhard Büdel, Universitat Kaiserslautern, Germany) in the laboratory of Algal Biotechnology, Bioscience Department, Barkatullah University, Bhopal, by standard microbiological techniques. The Antarctic sample came from Beacon sandstone boulder in the Linneaus Terrace (McMurdo Dry Valley) on the NNE flank of the Apocalypse peak. The hot desert sample came from a sandstone endolith from Arizona Coconino, 20 km from the Grand Canyon National Park U.S.A. Other details of the rocks relevant to the study are given in Table 1.

Table 1. Some features of the rocks from where the microbial communities were sampled.

Variable	Antarctic	Arizona
Dominant phototroph	*Chroococcidiopsis*	*Chroococcidiopsis*
pH	9.2	9.0
FRP (μg g^{-1})*	nd	11.36
TP (μg g^{-1})**	nd	883
TOP (μg g^{-1})***	nd	871
Chlorophyll (μg g^{-1})	7.56	9.22

*FRP=Filterable reactive phosphorus.
**=Total phosphorus.
***=Total organic phosphorus.

Culture Conditions

The cultures were maintained in modified CHU-10 (CHU10-D) medium as given by Chu [6] and modified by Gibson and Whitton [15]; P was reduced to 1 mgl^{-1}, EDTA used as a chelator, and pH buffered to 7.6 with HEPES. Ammonium -N (1 mgl^{-1}) was used as the nitrogen source in place of nitrate. Cultures were maintained at 20±2°C and a light flux of 60-70 μmol photons $m^{-2}s^{-1}$ and a light and dark cycle of 14:10 h. The culture used for experimental studies was grown for six months under the above-mentioned conditions to acclimatize the organisms to its new environment in the laboratory before starting the experiments. Also, as these are extremely slow growing organisms, this time period was needed to generate the cell mass required for the experiments. One set of cultures was kept at 5°C and 8 μmol photon $m^{-2}s^{-1}$ in an Orbital Shaking Incubator, representing conditions likely to occur in the Antarctic, and the other set from Arizona was kept at 40°C and 10 μmol photon $m^{-2}s^{-1}$ to ensure that both the isolated organisms could still grow in culture under the probable natural conditions in which they are found. These data have been obtained from scientists who have collected the rock samples. The light source for low light conditions was a 15-W fluorescent Phillips bulb fitted inside the Orbital shaker.

Measurement of Growth

Growth was measured by chlorophyll *a* extraction in 100% methanol. The amount of chlorophyll extracted was calculated by the equation of Mackinney [17]. The generation time was calculated by the growth equation of Kratz and Myers [16]. Different pH levels were maintained using a Systronics μ pH meter system 361. Different temperature ranges were maintained in different temperature-controlled Orbital Shaking Incubators.

Phosphatase Assay

Phosphomonoesterase (PMEase) activity was assayed routinely using a fluorometric method with the fluorogenic substrate 4-methylumbelliferyl phosphate (MUP), as this is considered to be the most sensitive method for this enzyme assay. The practical details are previously described [3, 28]. As the cultures were grown in very low concentrations of phosphorus in the medium (1 mgl^{-1}) initially, they were transferred to a P minus medium before the assay for two weeks to deplete the cells of phosphorus before the assay. Assays were

carried out in a P-free version of the CHU-10 D assay medium described by Mahasneh *et al.* [18]. The effect of pH on PMEase activity was carried out in a medium that was buffered to give a range of pH values using 100 µM final concentration of DMG (3,3-dimethyl-glutaric acid) for (pH 4.5- 5.5), HEPES (N-(2 hydroxyethyl) piperazine-N'-(2 ethanesulphonic acid) for (pH 6.0- 7.5), and glycine for (pH 8.0- 10). After buffering, each specific pH from acidic to alkaline range was checked and maintained with a Systronics µ pH meter system 361. Different temperatures used to study PMEase activity were maintained as described for growth. Wrapping the universal tubes with aluminum foil created dark conditions. Cell immobilization was done by the cell entrapment method using calcium alginate [26]. Assays were conducted in a water bath with gentle shaking. Temperature was measured at the beginning and end of the experiments to ensure that there was no difference between light and dark, especially for the Arizona samples where high temperatures were involved. A 0.1 ml aliquot of substrate from a stock solution of 600 µM was added to the universal bottles containing the required culture and buffer (total volume 6 ml) to give a final concentration of 100 µM for routine assays. Two controls with assay medium were sampled at each time interval along with the experimental ones: substrate but no alga, and alga but no substrate to ensure the presence of no other source of fluorescence. For immobilization, 4 beads and 0.2 ml culture had the same chlorophyll content and were used for the experiments as such. After the assay, the samples were passed through a GF/C filter and activity ended by using 10% (v/v) of the correct base/acid terminator. Using excitation at 356 nm, fluorescence emission was measured at 444 nm. The filter paper with the alga was dried for 24 h in a vacuum oven at 105°C. For immobilized cells, the 4 beads without alga were dried along with the beads containing the alga. The difference between the two gave the dry weight of the alga. The results are expressed as product (MU) formed g^{-1} d.wt h^{-1} (MU=4 methylumbelliferone). All results are mean±standard deviation of three independent replicates.

Phosphate Analysis

FRP (Filterable reactive phosphate-P) was measured using the method of Eisenreich *et al.* [9]. Total P was also measured after extraction from the rock samples using the digestion mixture as in of Eisenreich *et al.* [9].

Chlorophyll *a* Analysis

Chlorophyll *a* was extracted from rocks containing the *Chroococcidiopsis* community by the methanol extraction method previously described [19].

Statistical Analysis

The mean values and standard deviation are given in the figures as error bars. To determine the significance of differences between treatments, a t-test with a significance level of 0.05 was performed.

RESULTS

Table 1 shows some of the properties of the rock samples from where the two cultures of *Chroococcidiopsis* were obtained. For ease of description and tabulation, the Antarctic culture has been referred to as *Chroococcidiopsis* 1 and the Arizona culture has been referred to as *Chroococcidiopsis* 2 in the rest of the text.

Three standard culture media commonly used for cyanobacterial research (Foggs, CHU-10, BG-11) were used to test the growth of both cultures of *Chroococcidiopsis* to determine which culture medium was the most suitable for further experiments.

Figure 1 shows the generation time of the two cultures tested in the three media and fastest growth was recorded with CHU-10 for both. The generation time was 96 h and 85 h for *Chroococcidiopsis* 1 and *Chroococcidiopsis* 2, respectively. Effect of different temperatures (5- 50°C) on growth showed lowest generation time of 96 h and 85 h at 20±2°C and 40±2°C for *Chroococcidiopsis* 1 and 2. This value for *Chroococcidiopsis* 1 was significantly higher (p= <0.01) at 20°C compared to 5°C. In *Chroococcidiopsis* 2, however, 40±2°C showed higher value (p=<0.01) compared to 20±2°C. The effect of pH was also studied on the growth of the two cultures. The pH optima for maximum growth were 9.5 and 8.5 for *Chroococcidiopsis* 1 and 2, respectively. This pH was nearly the same as the rocks from where they were isolated (see Table 1). When the effect of dark condition was compared with light condition on growth of the two cultures, dark conditions produced negligible growth and no growth in organisms 1 and 2, respectively

Figure 2 shows the effect of different pH values on PMEase activity of both cultures. The pH optima were 9.5

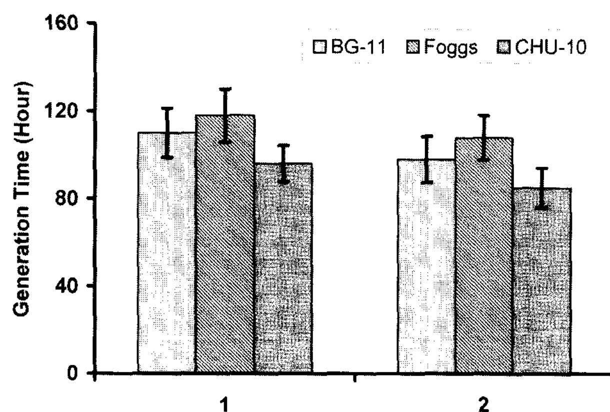

Fig. 1. Comparative growth of *Chroococcidiopsis* 1 and 2 shown as generation time in three different media.

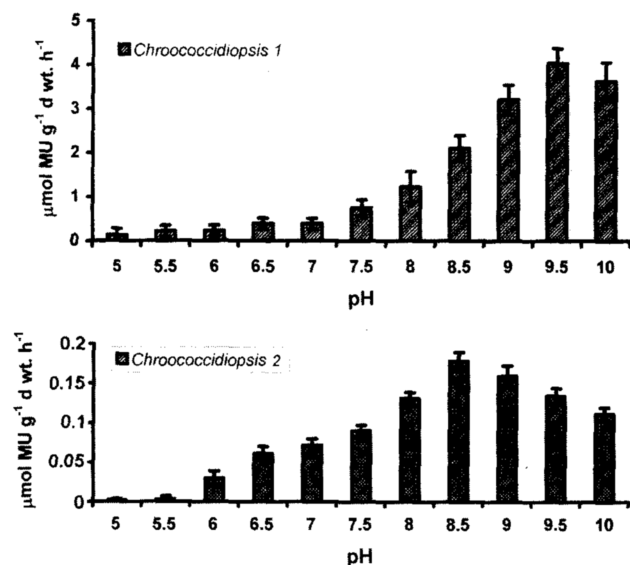

Fig. 2. Effect of pH on the PMEase activity of *Chroococcidiopsis* 1 and 2.

and 8.5 for *Chroococcidiopsis* 1 and 2, respectively ($p= <0.01$), compared to the acidic range. These values also supported maximum growth in both organisms and also recorded for the rock samples of the two habitats. Figure 3 shows the temperature optima for maximum PMEase

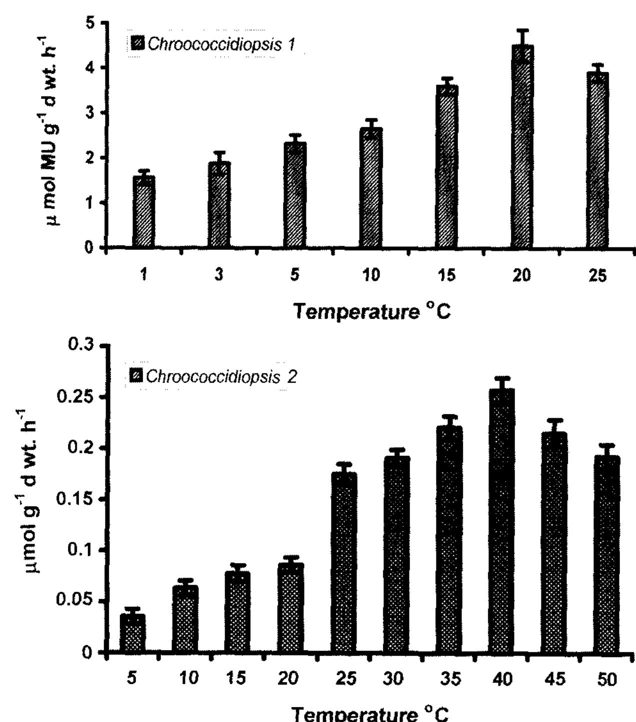

Fig. 3. Effect of temperature on the PMEase activity of *Chroococcidiopsis* 1 and 2.

Table 2. Comparison of optima for maximum PMEase activity of Antarctic and Arizona rocks, and cultures of *Chroococcidiopsis* isolated from them, together with their apparent K_m and V_{max} values. Assay condition of 8 µmol photon $m^{-2}s^{-1}$, substrate concentration of 100 µM MUP buffered at pH 9.5 by glycine for Antarctic, and 10 µmol photon $m^{-2}s^{-1}$, substrate concentration 100 µM MUP buffered at pH 9.5 by glycine for Arizona.

Sample	pH	Temperature	K_m (µM)	V_{max} (µmol MUg^{-1} h^{-1})
Antarctic rocks	9.2	5°C	230.8	0.053
Chroococcidiopsis-1	9.5	20°C	164.1	7.60
Arizona rocks	9.0	42°C	136.0	0.025
Chroococcidiopsis-2	8.5	40°C	132.8	0.021

activity of the two cultures, which were 20°C and 40°C for *Chroococcidiopsis* 1 and 2, respectively. Table 2 shows the comparison for maximum PMEase activity in Antarctic and Arizona rock samples dominated by *Chroococcidiopsis* which is the author's own work [2, 3], and the respective cultures isolated from them with apparent values of K_m and V_{max}. When the pH optima for the PMEase in both the Antarctic and Arizona rocks were compared with *Chroococcidiopsis* 1 and 2, the result in organism 1 was nearly the same (i.e. 9.5) but it differed slightly in the Arizona rock and organism 2. The temperature optima for maximum PMEase activity of Antarctic rock and culture were however found to be very different with rocks at 5°C and cultures at 20°C ($p= <0.01$). In contrast the values for Arizona rocks and samples were quite similar (40°C and 42°C). From the K_m and V_{max} values, it appears that PMEase activity of Antarctic rocks had a very high affinity for substrate while the isolate from it showed a low affinity in spite of the fact that the cultures were grown in very low concentrations of P in the medium in initial stages and then transferred to P-minus medium before the assay. Arizona rock and culture showed significant and similar affinity of PMEase for the substrate.

Table 3 shows the comparison of PMEase activity in light and dark of Antarctic and Arizona rocks and cultures of *Chroococcidiopsis* 1 and 2. Assuming that very little light reaches the endolithic layer under natural conditions because of its depth within the rock, it was desirable to test the effect of darkness on phosphomonoesterase activity.

Table 3. Comparison of PMEase activity of Antarctic and Arizona rocks and cultures of *Chroococcidiopsis* isolated from them in light and dark at 96 h. Assay condition of 8/10 µmol photon $m^{-2}s^{-1}$, substrate concentration of 100 µM MUP buffered at pH 9.5 by glycine.

Sample	Condition	Fold increase
Antarctic culture	Light	2.10
Arizona cultures	Light	1.85
Antarctic rocks	Dark	1.8
Arizona rocks	Light	2.06

Table 4. Comparative observations of optima of PMEase activity of immobilized culture of *Chroococcidiopsis* 1 and rock samples of the Antarctic. Assay condition of 8 μmol photon $m^{-2}s^{-1}$, substrate concentration of 100 μM MUP buffered at pH 9.5 by glycine.

Samples	pH	Temperature	Fold increase
Antarctic rocks	9.5	5°C	1.8
Immobilized culture	9.5	6.2°C	1.68

This experiment gave interesting results, where the Antarctic rocks showed much higher activity in the dark compared to light. Antarctic rocks showed 1.8-fold increase in dark over light in PMEase activity (p=<0.01). Cultures of *Chroococcidipsis* 1 however showed higher PMEase activity in light, and dark conditions were found to be inhibitory (p=<0.01). For the Arizona rocks and *Chroococcidiopsis* 2, this dark stimulation effect was not documented. When the PMEase activity of free and immobilized cells of *Chroococcidipsis* 1 were studied in light and dark, very interesting results were obtained with free cells showing 2.10-fold increase in PMEase activity in light over dark (p=<0.01). In contrast, immobilized cells of *Chroococcidiopsis* 1 showed 1.68-fold increase in PMEase activity in dark over light (p=<0.01). A comparison was then made between the immobilized *Chroococcidiopsis* 1 and the Antarctic rock samples containing the same organism. The Antarctic rocks had a pH optimum of 9.5, temperature optimum of 5°C, and 1.8-fold stimulation in the dark over light for PMEase activity. Immobilized cells of the *Chroococcidiopsis* 1 culture showed very similar results to the rock samples, having pH optimum of 9.5, temperature optimum of 6.2°C, and 1.68-fold increase in the dark over light (p=<0.01). In sharp contrast, although in the free cells pH optimum for PMEase activity was similar (i.e. 9.5), there was marked difference in temperature optimum (20°C) and a 2.10-fold increase in PMEase activity in light (Table 4). Therefore, it appears that PMEase activity of *Chroococcidiopsis* isolates from the Antarctic was quite similar to rock samples when immobilized.

DISCUSSION

The differing response obtained in cultured *Chroococcidiopsis* 1 is a very significant observation but it seems unlikely that such marked changes as observed for Antarctic whole cells and cultures in response to temperature and light could occur due to genetic drift. It seems more likely that it is an environmental response occurring within a few cell generations of the organism being removed from the rock and exposed to more favourable conditions. A similar suggestion was made by Fischer *et al.* [10] that the sensitivity to light of two immobilized cyanobacterial cultures was probably due to the thin layer of water in the immobilization chamber and their fine distribution on the surface just like the naturally immobilized *Chroococcidiopsis*. It seems likely that substantial portions of total P present in rocks or soil system are in organic form and are derived from decaying or lysed microorganisms and comprise a large portion of P uptake in such endolithic systems apart from the organic P released from the rocks. Therefore, phosphatase activity is the key to metabolism of P for this organism inside the rocks and the same holds true for their cultures. The observation with hot desert culture showing high phosphatase activity at temperatures as high as 42°C focus on the significance of adaptation of these enzymes to higher temperature and reflects the importance of flexibility of extreme values which could denature enzymes in nature.

The dark effect and other characteristic such as low temperature adaptation of phosphatase seem to be unique features of Antarctic rocks and are by no means universal, and this is also shown by immobilized cultures. The observation that a flux value as low as 8 μmol photons $m^{-2}s^{-1}$ is inhibitory suggests that even this low value may be above the optimum not only for PMEase activity of endolithic communities but other metabolic processes of the *Chroococcidiopsis* when immobilized under laboratory conditions. The precise reason for the difference in thermal responses of immobilized *Chroococcidiopsis* 1 isolate and the same organism within the rocks of the Antarctic may be due to the ability of immobilized cells to undergo a change which protects the PMEase from becoming nonfunctional at low temperatures. Low temperatures may also initiate *de novo* synthesis of certain proteins of a cryoprotective nature, which safeguards the enzyme from damage caused by freezing or subfreezing temperatures. Accumulation of disaccharide sugars is common in desert cyanobacteria like *Chroococcidiopsis* sp. [23] and it is probably this ability that allows not only the naturally occurring organism but also the culture to survive under dark conditions. These phenomena need more detailed explanation, not only because of its intrinsic interest but also for understanding the properties of organisms in the Antarctic dry desert so near the limits of life. It will also help to understand the properties of these organisms that might be used in studies such as those of Fischer *et al.* [10] and perhaps especially in their use in biosensors.

Acknowledgments

The authors are grateful to late Dr. D. D. Wynn Williams, Dr. Charles Cockels of British Antarctic Survey, U.K., for the Antarctic rock samples and Dr. Bukhard Büdel of Universitat Kaiserslautern, Germany for rock samples of the Arizona, Prof. Brian Whitton, Durham University for introducing the subject and for guidance, and Prof A. K.

Raghuwanshi Head and Dean, Life Science Faculty, Barkatullah University, Bhopal for providing necessary facilities in the Department. MB is grateful to the UGC, New Delhi, for the Grant of the Research Award.

REFERENCES

1. Avramescu, A., R. Rouillon, and R. Carpentier. 1999. Potential for use of a cyanobacterium *Synechocystis* sp. immobilized in poly vinyl alcohol: Application to the detection of pollutants. *Biotechnology Techniques* **13(8):** 559- 562.
2. Banerjee, M., B. A. Whitton, and D. D. Wynn-Williams. 2000. Surface phosphomonoesterase activity of a natural immobilized system: *Chroococcidiopsis* in an Antarctic desert rock. *J. Appl. Phycol.* **12:** 549- 552.
3. Banerjee, M., B. A. Whitton, and D. D. Wynn-Williams. 2000. Phosphatase activities of endolithic communities in rocks of Antarctic dry valleys. *Microb. Ecol.* **39:** 80- 91.
4. Bell, R. A. and M. R. Sommerfield. 1987. Algal biomass and primary production within a temperature zone sandstone. *Am. J. Bot.* **74:** 294- 297.
5. Büdel, B., U. Luttge, R. Stelzer, O. Huber, and E. Medina. 1994. Cyanobacteria of rocks and soil of the Orinoco lowlands and the Guayana uplands, Venezuela. *Botanica Acta* **107:** 422- 431.
6. Chu, S. P. 1942. The influence of mineral composition of the medium on the growth of planktonic algae. I. Methods and culture media. *J. Ecol.* **30:** 284- 325.
7. Cockell, C. S., P. Rettberg, G. Horneck, M. R. Patel, H. Lammer, and C. Cordoba-Jabonero. 2002. Ultrviolet protection in micro-habitats; lessons from the terrestrial poles applied to Mars. *ESA SP* **518:** 215- 218.
8. de la Torre, J. R., B. M. Goebel, E. L. Friedmann, and N. R. Pace. 2003. Microbial diversity of cryptoendolithic communities from the McMurdo dry valleys Antarctica. *Appl. Environ. Microbiol.* **69(7):** 3858- 3867.
9. Eisenreich, S. J., R. T. Bannerman, and D. E. Amstrong. 1975. A simplified phosphorus analysis technique. *Environ. Lett.* **9:** 43- 53.
10. Fischer, D., U. G. Schlosser, and P. Pohl. 1997. Exopolysaccharide production by cyanobacteria grown in enclosed photobioreactors and immobilized using white cotton towelling. *J. Appl. Phycol.* **9:** 205- 213.
11. Friedmann, E. I. 1978. Melting snow in the Dry Valley is the source of water for endolithic microorganisms. *Antarct. J. U. S.* **13:** 162- 163.
12. Friedmann, E. I. 1982. Endolithic microorganism in the Antarctic cold desert. *Science* **215:** 1045- 1053.
13. Friedmann, E. I. and R. Ocampo. 1976. Endolithic blue green algae in the Dry Valley. Primary producers in the Antarctic desert ecosystem. *Science* **193:** 1247- 1249.
14. Friedmann, E. I., A. Y. Druk, and C. P. Mckay. 1994. Limits of life and microbial extinction in the antarctic desert. *Antarct. J. U. S.* **29:** 176- 179.
15. Gibson, M. T. and B. A. Whitton. 1987. Influence of phosphorus on morphology and physiology of fresh water Chaetophora, Drapamaldia and Stigeoclonium (Chaetophorales, chlorophyta). *Phycologia* **26:** 59- 69.
16. Kratz, W. A. and J. Myers. 1955. Nutrition and growth of several blue green algae. *Am. J. Bot.* **42:** 282- 287.
17. Mackinney, G. 1941. Absorption of light by chlorophyll. *J. Biol. Chem.* **140:** 315- 322.
18. Mahasneh, I. A., S. L. J. Grainger, and B. A. Whitton. 1990. Influence of salinity on hair formation and phosphatase activity of the blue green alga (Cyanobacterium) *Calothrix viguieri* D253. *Br. Phycol. J.* **25:** 25- 32.
19. Marker, A. F. H. 1995. *Chlorophyll Analysis, Standard Methods*. National Rivers Authority, Bristol, U.K.
20. McKay, C. P., E. I. Friedmann, B. Gomez-Silva, L. Caceres-Villanueva, D. T. Anderson, and R. Landheim. 2003. Temperature and moisture conditions for life in the extreme arid region of the Atacama desert: Four years of observations including the EL Nino of 1997- 1998. *Astrobiology* **3(2):** 393- 406.
21. Nienow, J. A. and E. I. Friedmann. 1993. Terrestrial lithophytic (rock) communities, pp. 353- 412. *In* E. I. Friedmann (ed.), *Antarctic Microbiology*. Wiley-Liss, New York, U.S.A.
22. Patel, M. R., A. Berces, C. Kolb, P. Rettberg, J. C. Zarnecki, and F. Selsis. 2003. Seasonal and diurnal variation in Martian surface UV irradiation: Biological and chemical implications for the Martian regolith. *Int. J. Astrobiol.* **2(1):** 21- 34.
23. Potts, M. 1994. Desiccation resistance of prokaryotes. *Microbiol. Rev.* **58:** 755- 805.
24. Romo, S. and C. Perez Martinez. 1997. The use of immobilization in alginate beads for long-term storage of *Pseudoanabaena galeata* (Cyanobacteria) in the laboratory. *J. Phycol.* **33:** 1073- 1076.
25. Ronto, G., A. Berces, H. Lammer, C. S. Cockell, G. J. Molina-Cuberos, M. R. Patel, and F. Selsis. 2003. Solar UV irradiation conditions on the surface of Mars. *Photochem. Photobiol.* **77(1):** 34- 40.
26. Singh, S. P., S. K. Verma, R. K. Singh, and P. K. Pandey. 1989. Copper uptake by free and immobilized cyanobacterium. *FEMS Microbiol. Lett.* **60:** 193- 196.
27. Vincent, W. F. 1988. *Microbial Ecosystems of Antarctica*. Cambridge University Press, Cambridge Press, pp. 304.
28. Whitton, B. A., S. L. J. Grainger, G. R. W. Hawley, and J. W. Simon. 1991. Cell bound and extracellular phosphatase activities of cyanobacterial isolates. *Microb. Ecol.* **21:** 85- 98.
29. Wilkinson, S. C., K. H. Goulding, and P. K. Robinson. 1990. Mercury removal by immobilized algae in batch culture systems. *J. Appl. Phycol.* **2:** 223- 230.
30. Williams, D. D. 2000. Cyanobacteria in desert-life at limits, pp. 341- 366. *In* Whitton, B. A. and M. Potts M (eds.), *Ecology of Cyanobacteria: Their Diversity in Time and Space*. Kluwer, Dordrecht.
31. Wynn-Williams, D. D., N. C. Russell, and H. G. M. Edwards. 1997. Moisture and habitat structure as regulator for microalgal colonists in diverse Antarctic terrestrial habitats, pp. 77- 88. *In* W. B. Lyons, C. Howard-Williams, and I. Hawes (eds.), *Ecosystems in Antarctic Ice free Landscapes*. Balkema, Rotterdam.