REGULATION OF PHOSPHATASE ACTIVITY IN CHROOCOCCIDIOPSIS ISOLATES FROM TWO DIVERSE HABITATS: EFFECT OF LIGHT, PH AND TEMPERATURE.

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Abstract: Phosphomonoeaterase (PMEase) and phosphodiesterase (PDEase) activity was studied in the cyanobacterial cultures of Chroococcidiopsis isolated from two diverse cryptoendolithic habitats of Antarctic and Arizona. Because this organism is found within the rocks it appears that phosphorus metabolism by alkaline phosphatase activity is a key factor to sustain growth of these organisms in that state, there being no other source of external P except in bound form in the rocks. The main findings in this paper show that specific pH and temperature regulate the PMEase and PDEase activities studied with different substrates in isolated of Chroococcidiopsis 1 and 2 although values were vastly different. The pH and temperature optima for phosphatase activity (PMEase and PDEase) of Chroococcidiopsis 1 and 2 were 9.5, 20 °C and 8.5, 40 °C respectively. It needs mentioning here that although the pH optimum for the enzyme activities in the Antarctic rock samples was the same i.e. 9.5 there was a striking difference in the temperature optimum in which maximum activity of both enzymes were recorded at 5 °C. A very crucial role of light and dark conditions were important for the enzyme activity and differed to a significant extent when compared with naturally occurring organisms in the Antarctic rocks. Low light fluxes of about 8 µmol photon $m^{-2}s^{-1}$ showed higher PMEase and PDEase activity then total dark conditions in the Chroococcidiopsis -1 culture. However under natural conditions when this organism is found within the rocks 8-µmol photon $m^{-2}s^{-1}$ was found to be inhibitory and dark conditions gave higher PMEase and PDEase activity. Arizona rock samples containing Chroococcidiopsis -2 however did not show dark stimulation. Increase in light intensity from 8 µmol photon $m^{-2}s^{-1}$ to 60 µmol photon $m^{-2}s^{-1}$ maintained in the culture room increased the PMEase and PDEase activity of both cultures. The unique light and temperature responses for PMEase and PDEase activities in Chroococcidiopsis-1 found within the Antarctic rocks are unique. It points to some change in the cells probably by producing some cryoprotectant which protects the enzymes from becoming non functional at low temperatures. It also indicates that exposure to light fluxes as low as 8 µmol photon $m^{-2}s^{-1}$ can probably alter the properties of the enzymes thus reducing its activity compared to total darkness.
Keywords: *endolithic, cyanobacteria, phosphomonoesterase, Antarctic, Arizona*

Introduction

The Ross desert of the McMurdo dry valley, Antarctica 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 [23, 26, 25]. Areas of extensive rock exposure represent one of the harshest environments of our planet. Examples of other such extreme habitats are the hot deserts like Arizona USA. The surface of these hot and cold deserts is practical-

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ly abiotic and communities of indigenous life forms if present are hidden within the cracks and depressions as cryptoendoliths. Microorganisms that occupy a sharply defined zone colonize the communities existing under the surface of the rocks. The dominant organism in these habitats are the cyanobacteria [20, 22, 12, 25]. Among the cyanobacterial species the most dominant form is the unicellular cyanobacterium Chroococcidiopsis, and is found to grow where all other organisms fail to grow and is the most primitive organism [2, 9, 10, 11].

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 [1, 2, 4, 25, 26, 12, 20, 7].

Most studies on nutrient uptake and cycling in endolithic communities of hot and cold deserts have dealt with carbon (3, 19] and there are no reports on phosphorus and nitrogen metabolism. Results on whole communities (rock samples) of the Antarctic [1, 2] 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 isolates 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 [6, 7, 10, 11, 25]

We therefore decided to study the PMEase and PDEase activity of Chroococcidiopsis isolated from two extreme habitats of hot and cold deserts of Antarctic and Arizona using different substrates. Also an attempt has been made to see the regulation of the enzyme activities with differing pH, temperature and light regimes under laboratory conditions and a comparison has been made with natural habitats, which is the author's own work.

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, USA 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 USA. Other details of the rocks relevant to the study are given in *Table 1.*

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

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

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

Table 2. *Comparison of rates of PMEase and PDEase activities of Chroococcidiopsis 1 using 100 mM of different substrates. Rates are expressed as product (mmol pNP, MU, bis-pNP) released per hour ±SD (n=3). Experiments have been carried out at 20 °C and 275 Lux light intensity. (0 hour activity was 0.21±0.002)*

Time in hours	PNPP	MUP	Bis-pNPP
2 L	7.53±0.48	4.06±0.68	2.86±0.77
2D	3.22±0.12	1.93±0.12	1.86±0.31
4L	15.85±1.13	8.86±1.0	6.88±1.21
4D	7.12±1.11	3.26±0.78	2.79±0.22
8L	22.1 ±3.2	11.98±2.3	9.78±1.67
8D	4.3 ±0.78	2.33±0.88	2.03±0.55
12L	13.66±2.6	13.66±2.6	11.60± 2.01
12D	1.23±0.33	1.23±0.33	0.98±0.02

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Culture conditions

The cultures were maintained in modified CHU-10 (CHU10-D) medium as given by, [5] and modified by [13]; P was reduced to 1mgl^{-1}, EDTA used as a chelator , and pH buffered to 7.6 with HEPES. Ammonium -N (1mgl^{-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 hrs. The material used for experimental studies was grown for six months under the above-mentioned conditions to acclimatize the organism 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 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.

Phosphatase Assay

Phosphomonoesterase (PMEase) activity was assayed routinely using the fluorometric method with the fluorogenic substrate 4-methylumbelliferyl phosphate (MUP) and the calorimetric method using para-nitro-phenyl phosphate (pNPP). Phosphodiestrase activity was assayed using Bis pNPP calorimetrically. The practical details are given in [24, 2].

As the cultures were grown in very low concentrations of P in the medium (1mgl^{-1}) initially they were transferred to a P minus medium before the assay for two weeks to deplete the cells of P. Assays were carried out in a P free version of the CHU-10 D assay medium described in [17]. The effect of pH on PMEase activity was carried out in a medium that was buffered to give a range of pH values using 100mM 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) for effect of pH on PMEase activity. After buffering, each specific pH from acidic to alkaline range was maintained and checked with a Systronics m pH meter system 361. Different temperature ranges were maintained in a Remi Instrument, temperature controlled Orbital Shaking Incubator and other incubators. Wrapping the universal tubes with aluminum foil created dark conditions. 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 mmol was added to the universal bottles containing the required cultures 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/ colour development. 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 444nm. For spectrophotometric analysis absorbance was measured by recording the optical density in a

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Systronic spectrophotometer model-169 at 405 nm The filter paper with the alga was dried for 24 hours in a vacuum oven at 105 °C. The results are expressed as product (MU/pNP,/Bis pNP) formed g^{-1} d.wt h^{-1}. (MU = 4 methylumbelliferone, pNP= paranitrophenol, Bis-pNP=Bis para nitrophenol). All results are mean ± standard deviation of three independent replicates. Individual experiments have been conducted with MUP as the substrate as it is the most sensitive method of phosphatase activity measurement while comparative studies have been conducted with all three substrates.

Phosphate Analysis

FRP (Filterable reactive phosphate-P) was measured using the method in [8]. Total P was also measured after extraction from the rock samples using the digestion mixture as in [8].

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 as Chroococcidiopsis-1 and the Arizona culture has been referred as Chroococcidiopsis-2 in the rest of the text.

Table 2 shows the comparative time course studies on PMEase and PDEase activity of Chroococcidiopsis -1 using MUP and pNPP as PMEase substrate and Bis-pNPP as PDEase substrate at light low light intensities of 8 mmol photon $m^{-2}s^{-1}$ as probably found in the Antarctic endoliths. Unlike the rocks samples it was found that even this low light intensity was stimulatory for both enzymes compared to dark conditions ($p = < 0.01$). With increase in time the light driven activity increased steadily, while the dark activity decreased drastically after the fourth day. The substrate pNPP gave higher activity of PMEase compared to MUP and PDEase activity was nearly half that of the PMEase activity. *Table 3* shows the comparative PMEase activity and PDEase activity of Chroococcidiopsis 2 using low light intensities as the probable Arizona endolithic conditions. Here similar to the rock samples higher PMEase and PDEase activity was obtained with cultures in low light compared to dark conditions ($p = < 0.05$). The substrate pNPP gave higher activity of PMEase compared to MUP and PDEase activity was nearly half that of the PMEase activity. Experiments were also conducted in normal culture room light (60 mmol photon $m^{-2}s^{-1}$) to study the PMEase and PDEase activities of isolate 1 to see the variations if any in higher light intensities. It was found that the activity increased to a great extent under these conditions in all three substrates use (*Table 4*). Very similar observations were obtained in isolate 2 but here the rates were higher than in isolate 1 (*Table 5*). *Table 6* shows the percent increase of PMEase activity in light and dark of Antarctic and Arizona rocks and cultures of Chroococcidiopsis 1 and 2. Antarctic rocks showed 51.1 percent increase in dark over low light in PMEase activity ($p = < 0.01$). Cultures of Chroococcidipsis 1 however showed higher PMEase activity in low light (53.2 percent) and dark conditions were found to be inhibitory ($p = < 0.01$). For the Arizona rocks and Chroococcidiopsis 2 the percent increase was nearly the same (49.2 and 50.2 percent) in low light conditions compared to dark and dark stimulation effect in rock samples was not documented. *Figure 1* shows the percent increase in PMEase and PDEase activity of Chroococcidiopsis 1 and 2 compared to low light inten-

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sities (8 and 60 mmol photon $m^{-2}s^{-1}$) using MUP as a substrate. There was considerable increase in PMEase ($p = < 0.01$) and PDEase ($p = < 0.05$) activity in both isolates with increase in light intensity but it was higher in isolate 2. *Figure 2* shows the percent increase in PMEase and PDEase activity of Chroococcidiopsis 1 and 2 when high light intensities (60 mmol photon $m^{-2}s^{-1}$) are compared with dark conditions using MUP as a substrate. In this case there was greater increase in PDEase activity in both organisms compared to PMEase ($p = < 0.05$).

Figure 3 shows the effect of different pH values on PMEase activity of both cultures using MUP as the substrate. The pH optima were 9.5 and 8.5 for Chroococcidiopsis 1 and 2 respectively ($p = < 0.01$) compared to the acidic range. These values were recorded for the rock samples of the two habitats. *Figure 4* shows the temperature optima of PMEase activity of the two cultures using MUP as substrate. It was 20 °C for Chroococcidiopsis 1 and 40 °C for Chroococcidiopsis 2. *Table 7* shows the comparison for maximum PMEase activity in Antarctic and Arizona rock samples dominated by Chroococcidiopsis values of which are available, Banerjee et al (2000a) and the respective cultures isolated from them with apparent values of K_m and V_{max}. When the pH optima for the enzyme 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. 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 for the substrate.

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. 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 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 good for their cultures. The higher activity of pNPP compared to MUP as a substrate in the present study is probably because it has been shown that hydrolysis of pNPP can be brought about by a range of enzymes that are present in the cells and not just PMEase [16]. The significantly less PDEase activity compared to PMEase activity in this study was similar to results obtained for cultured cyanobacteria [15]. This is probably because the PDEase releases about 25% of the pi from Bis-pNPP compared to that released by pNPP. The monoesterases are capa-

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Table 3. Comparison of rates of PMEase and PDEase activities of Chroococcidiopsis 2 using 100 mM of different substrates. Rates are expressed as product (mmol pNP, MU, bis-pNP) released per hour ±SD(n=3). Experiments have been carried out at 40 °C and 275 Lux light intensity. (0 hour activity was 0.25±0.001)

Time in hours	pNPP	MUP	Bis-pNPP
2 L	9.12±1.48	3.10±0.65	4.62±0.71
2D	2.26±0.72	1.8±0.17	1.88±0.34
4L	16.12±1.23	6.2±1.05	7.51±1.01
4D	4.98±1.01	2.93±0.87	2.33±0.25
8L	20.06 ±2.9	8.37±1.3	10.24±1.17
8D	3.24 ±0.88	1.06±0.18	1.56±0.45
12L	22.42±3.6	11.35±2.7	11.11± 2.13
12D	1.63±0.35	0.80±0.03	0.94±0.02

Table 4. Comparison of rates of PMEase and PDEase activities of Chroococcidiopsis 1 using 100 mM of different substrates. Rates are expressed as product (mmol pNP, MU, bis-pNP) released per hour ±SD (n=3). Experiments have been carried out at 20 °C and 2,500 Lux light intensity. (0 hour activity was 0.21±0.002)

Time in hours	pNPP	MUP	Bis-pNPP
2 L	10.68±1.37	8.9±0.95	4.96±0.91
4L	17.32±1.56	13.3±1.05	8.22±0.99
8L	23.06 ±3.2	18.1±1.3	11.59±1.17
12L	26.22±3.6	20.6±2.6	14.06±2.13

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ble of providing the necessary levels of phosphorus to the cells as the substrates are easily available and energetic related to the reactions less compared to diesterases and their activity therefore occurs at higher rates as observed.

The higher percent increase in PDEase activity when effect of high light intensities was compared to dark is probably due to the general observation that PDEase activity was greatly reduced in the dark compared to low as well as high light intensities.

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. The observation that a flux value as low as 8 µmol photons $m^{-2}s^{-1}$ is inhibitory suggests that even this low value maybe above the optimum not only for PMEase activity of endolithic communities. These organisms might be possessing special accessory pigments for harvesting PAR or the chloroplasts and/or thylakoids of the cryptoendoliths may be morphologically adapted to scavenge the lowest limit of available photons. It is also possible that dark exposure may lead to some change in the wall or enzyme structure which renders the active sites more accessible to the substrate. The precise reason for the difference thermal response of the Chroococcidiopsis –1 and the Antarctic rocks containing the same organism may be due to the ability of naturally immobilized cells in the rocks to undergo a change which protects the enzyme from becoming non functional at low temperatures.

Low temperature may also initiate de novo synthesis of certain proteins that are cryoprotective in nature which safe guards the enzyme from damage caused by freezing or sub freezing temperatures. A suggestion similar to this was given by Guy [14]. 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.

Table 5. *Comparison of rates of PMEase and PDEase activities of Chroococcidiopsis 2using100 mM of different substrates. Rates are expressed as product (m mol pNP, MU,bis-pNP) released per hour ±SD (n=3). Experiments have been carried out at 40 °C and 2,500 Lux light intensity. (0 hour activity was 0.25±0.001)*

Time in hours	pNPP	MUP	Bis-pNPP
2 L	12.06±1.77	9.38±1.95	6.73±1.91
4L	19.19±2.06	15.19±1.25	9.75±1.59
8L	24.16 ±3.4	19.1±2.3	12.09±1.67
12L	27.28±3.67	20.6±2.8	13.66±2.73

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Table 6. *Percent increase in PMEase activity of Antarctic and Arizona rocks and cultures of Chroococcidiopsis isolated from them in two light conditions and dark at 96h. Percent increase has been calculated with light compared to dark and dark compared to light as the case may be and as indicated in the conditions.*

Sample	Condition	Percent increase
1.Antarctic culture*	Light	53.2
2 Arizona cultures*	Light	50.2
3. Antarctic rock*	Dark	51.1
4. Arizona rocks*	Light	49.2

Table 7. *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 8µ mol photon $m^{-2}s^{-1}$, substrate concentration 100 µM MUP buffered at pH 9.5 by glycine for Antarctic and 10 µ mol photon $m^{-2}s^{-1}$, substrate concentration 100 µM MUP buffered at pH 9.5 by glycine for Arizona.*

Sample	pH	Temperature	Km (µM)	V_{max} (µ mol 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

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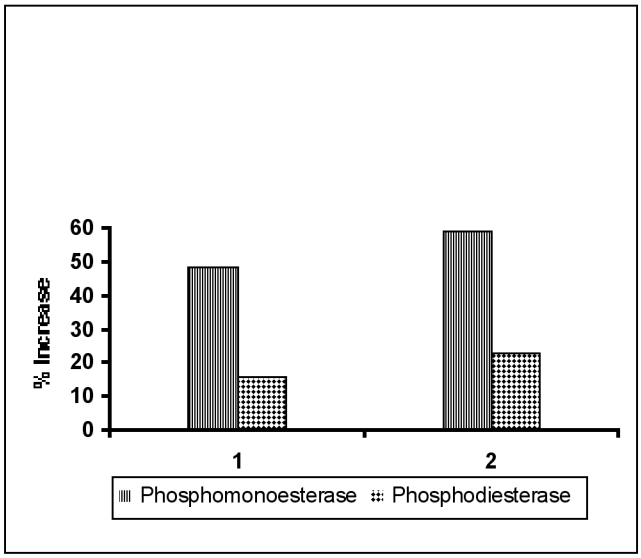

Figure 1. Percent increase in PMEase and PDEase activity of Chroococcidiopsis 1 and Chrococcidiopsis 2 when high Light intensity are compared to low light intensity (60 & 8 mmol photon $m^{-2}s^{-1}$).

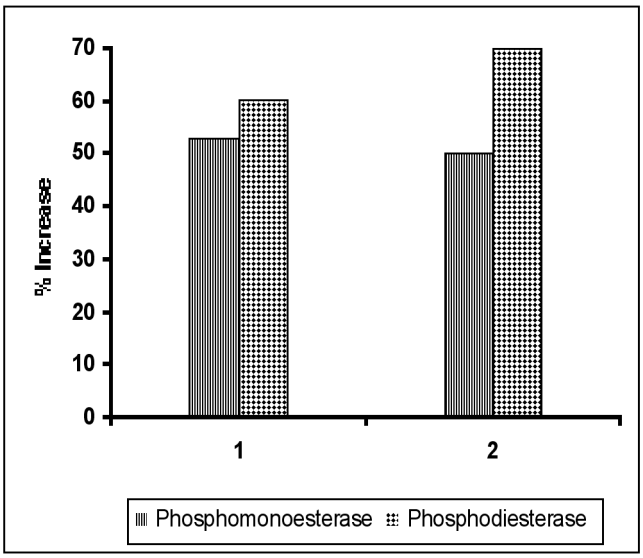

Figure 2. Percent increase in PMEase and PDEase activity of Chroococcidiopsis 1 and Chrococcidiopsis 2 when high light intensity (60 mmol photon $m^{-2}s^{-1}$) are compared to dark conditions.

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Accumulation of disaccharide sugars is common in desert cyanobacteria like Chroococcidiopsis sp. [21] and it is probably this ability that allows not only the naturally occurring organism but also the culture to survive under dark conditions. This phenomenon needs 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 and should prove to be a fertile area for research in the future.

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