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2.2 The Effect of Low Temperatures on Antarctic Endolithic Green Algae

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Summary: Laboratory experiments show that undercooling to about –5° C occurs in colonized Beacon sandstones of the Ross Desert, Antarctica. High-frequency temperature oscillations between 5° C and –5° C or –10° C (which occur in nature on the rock surface) did not damage *Hemichloris antarctica*. In a cryomicroscope, *H. antarctica* appeared to be undamaged after slow or rapid cooling to –50° C. $^{14}CO_2$ incorporation after freezing to –20° C was unaffected in *H. antarctica* or in *Trebouxia* sp. but slightly depressed in *Stichococcus* sp. (isolated from a less extreme Antarctic habitat). These results suggest that the freezing regime in the Antarctic desert is not injurious to endolithic algae. It is likely that the freezing-point depression inside the rock makes available liquid water for metabolic activity at subzero temperatures. Freezing may occur more frequently on the rock surface and contribute to the abiotic nature of the surface.

Zusammenfassung: Laborversuche zeigen, daß in den besiedelten Teilen des Beacon-Sandsteins der Ross-Desert in der Antarktis Unterkühlungen bis –5° C vorkommen. Rasche Temperaturschwankungen zwischen 5° C und –5° C oder –10° C (wie sie in der Natur auf der Felsoberfläche vorkommen) schädigten *Hemichloris antarctica* nicht. *H. antarctica* erschien nach langsamer oder rascher Abkühlung auf –50° C ungeschädigt. Im Kryomikroskop erschien *H. antarctica* nach langsamer oder rascher Abkühlung bis –50° C unbeschädigt. $^{14}CO_2$-Aufnahme war nach Frieren bis –20° C nicht beeinflußt bei *H. antarctica* und *Trebouxia sp.*, aber leicht erniedrigt bei *Stichococcus sp.* (isoliert von einem weniger extremen antarktischen Standort. Diese Resultate zeigen, daß Fröste in der antarktischen Wüste die endolithischen Algen nicht schädigen. Wahrscheinlich ermöglicht eine Gefrierpunktserniedrigung innerhalb des Gesteins, daß auch bei Temperaturen unter Null tropfbares Wasser für den Stoffwechsel verfügbar ist. Außen auf den Gesteinen gefriert Wasser häufiger, und trägt so zur Lebensfeindlichkeit der Felsoberfläche bei.

1. INTRODUCTION

The abundance of cryptoendolithic microorganisms under a largely abiotic rock surface in the Ross Desert (the desert areas of the McMurdo Dry Valleys) of Antarctica suggests that the endolithic habitat is a refuge in a "hostile" environment. The yearly temperature extremes span over 60° C (in sloped rocks) with a minimum of approximately –50198 C (FRIEDMANN et al. 1987). Diurnal summer temperatures can range over 20° C resulting in a daily freeze-thaw cycle. Under certain weather conditions, high-frequency oscillations around 0° C (approximate periods of 3 and 10 min.) can occur at the rock surface while subsurface temperature fluctuations are damped and remain above freezing (FRIEDMANN et al. 1981, McKAY & FRIEDMANN 1985). It has been speculated that the high-frequency oscillations and the resulting rapid freeze-thaw cycles are responsible for the abiotic nature of rock surfaces in the Ross Desert.

Rapid fluctuations through 0° C do not necessarily result in high-frequency freeze-thaw cycles. Water can remain unfrozen at sub-zero temperatures for significant periods of time, if nucleating agents are not present and if the water is contained in a small volume or influenced by strong surface interactions (see FRANKS 1985). GREEVEY & WHALLEY (1982) reported that –3° C is a typical freezing point for limestone and sandstone.

Metabolic activity in the cryptoendolithic microbial community at temperatures below –5° C has been demonstrated (KAPPEN & FRIEDMANN 1983, VESTAL 1988). KAPPEN & LANGE (1970a) demonstrated the remarkable resistance of lichen phycobionts after exposure of the lichen to –78° C and subsequent isolation of the phycobiont. Based on measurements of CO_2 exchange, lichens can tolerate –15° C for nearly two years (LANGE 1966), and Antarctic lichens survive temperatures well below those encountered in the environment (KAPPEN & LANGE 1970b, LANGE & KAPPEN 1972). In contrast, the snow algae *Chlamydomonas nivalis, C. yellowstoniensis*, and *Chloromonas palmelloides*, which tolerate freezing to –10° C, show irreparable damage after exposure to –20° C (CLARKE & LEESON 1985). HOLM-HANSEN (1963) found a decrease in viability in six out of seven aquatic species of Antarctic green algae frozen to –10° C, and all seven were injured by freezing to –25° C, with the survival rate ranging from 13 to 82%. In experiments using successive freeze-thaw cycles, two soil isolates, *Bracteacoccus* sp. and *Stichococcus bacillaris*, showed sensitivity to freezing similar to that of the aquatic species tested.

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Two questions are addressed in this paper:
(1) What is the freezing point of water in the cryptoendolithic environment?
(2) What effect, if any, does the thermal regime have on cryptoendolithic algae?

The temperature at which water freezes in colonized rocks was determined empirically because the complexity of this system precludes theoretical prediction. The freezing sensitivity of Ross Desert cryptoendolithic eukaryotic algae was investigated in cultures subjected to freezing and thawing and compared for reproductive viability, cryomicroscopy, and CO_2 uptake.

2. MATERIALS AND METHODS

2.1 *Rock Freezing*

In experiments to determine the temperature at which water freezes inside a rock, exotherms (latent heat released) were detected by measurement of the temperature difference between insulated and uninsulated copper-constantan (type-T) thermocouples wired in series (differential-thermocouple) and embedded in rock. Both ends were embedded 0.5 cm deep into a 340 g piece of colonized Beacon sandstone rock from Linnaeus Terrace, Ross Desert, Antarctica. A bare type-T thermocouple was also embedded to measure actual rock temperature.

The monitored rock, packed in a styrofoam container, was placed in a –80° C freezer, producing an effective cooling rate of 0.1° C min^{-1} at high sub-zero temperatures. The following day, the container was allowed to warm to room temperature. Measurements were taken every 0.5 s, and the mean, maximum, and minimum for each two-minute interval were recorded by a datalogger (Campbell Scientific Inc., Logan, UT).

2.2 *Cultures*

Algal cells were grown in liquid Bold's Basal Medium (BBM), at 10° C, under a 16/8 hour light/dark cycle with 15—20 µmole photons m^{-2} s^{-1} provided by cool white fluorescent lights. The cultures of green algae used in these experiments were isolated by Dr. R. Ocampo-Friedmann and are maintained in the Culture Collection for Microorganisms from Extreme Environments (CCME) at Florida State University. Strain descriptions are listed in Table 1. *Hemichloris antarctica*, a free-living alga, is a frequent member of the cryptoendolithic community. *Trebouxia* sp. is a phycobiont of crypto- and chasmoendolithic lichens. *Stichococcus* sp. occurs frequently in the cryptoendolithic community and elsewhere in the Antarctic desert, although in very low abundance (FRIEDMANN et al. 1988). This particular strain, isolated from the surface of a lichen, is not endolithic and originates from a less extreme Antarctic habitat.

CCME No.	Organism	Origin
(126) A778-50	*Hemichloris antarctica* Tschermak-Woess & Friedmann	cryptoendolithic in sandstone, floor of Beacon Valley
(189) A790-21	,, ,,	cryptoendolithic in sandstone, Linnaeus Terrace
(188) A790-21	*Trebouxia* sp.	phycobiont of chasmoendolithic lichen in granite, Victoria Valley
(170) A789-89	*Stichococcus* sp.	surface of unidentified lichen growing on sandstone Forrestal Range

Table 1: Strain histories.

2.3 *Viability*

Cultures of *Hemichloris antarctica* (7 and 42 days old) were placed in an Endocal Digital Refrigerated Circulating Bath, and the thermal regime was controlled by a MTP-5 programmer (Neslab Instruments, Inc., Portsmouth, NH) to simulate the high-frequency oscillations occurring on rock surfaces. After the desired number of cooling and warming cycles, inocula were transferred into fresh BBM. Cell numbers were counted every three to four days for the following 28 days. The growth constant (K_c; GUILLARD 1973) was calculated for each three- or four-day period during which there was log-phase growth. Reproductive viability was determined on the assumption that differences in measured growth rate are due to the proportion of dead cells being counted and not to a change in the growth rate of the living cells themselves. The growth constant formula then becomes:

$$K_e = \frac{\ln ((N_1 + D_0) / (N_0 + D_0))}{(t_1 - t_0)}$$

N_1 and N_0 are the number of live cells at times t_1 and t_0. D_0 is the number of dead cells inadvertently counted. With this formula, the number of live cells can be calculated and the percent viability determined as the number of live cells (N_0) divided by the number of cells counted ($N_0 + D_0$). For comparison purposes, the controls are assumed to have 100% viable cells.

2.4 *Cryomicroscopy*

At the Culture Centre for Algae and Protozoa, U. K., *Hemichloris antarctica* (CCME 126) was observed during different freezing and thawing rates, under a cryomicroscope as described by MCGRATH (1987). The black-and-white micrographs for this paper were reproduced from the original color transparencies with a Kodak Wratten # 48 blue filter (FRIEDMANN 1966) to increase the contrast of the chloroplasts (see TSCHERMAK-WOESS & FRIEDMANN 1984).

2.5 *Carbon dioxide uptake*

Logarithmically growing cultures were pipetted into 5 ml culture tubes and sealed with parafilm. Control and frozen cells were filtered onto Nucleopore polycarbonate filters (0.4 µm pore size). The filters were placed on wetted glass-fiber filters within an air-tight glass chamber. After a 30-minute incubation at 10° C and 20 µmoles photons $m^{-2} s^{-1}$, the chamber's atmosphere was inoculated with radiolabeled CO_2. As a check that equal radiolabel concentrations were used for control and treatments, triplicate 0.25 ml air samples were taken and pipetted into 5 ml of liquid scintillation cocktail (Scinti Verse E) containing 0.5 ml of phenylethylamine. After two hours of incubation, the filters were washed with three 2 ml aliquots of distilled water, aspirated dry, and placed in 5 ml of liquid scintillation cocktail. The activity on the filters was measured by duplicate counts in a liquid scintillation counter.

3 RESULTS

3.1 *Freezing point*

Figure 1 is an example of the time course of temperature and the differential-thermocouple output during which a rock with 5 g of water is cooled through the freezing point. The large peak in the differential-thermocouple curve, as well as the rise in rock temperature, is the result of latent heat dissipated during ice formation.

No other exotherms were detected with a further drop in temperature. Upon warming, endotherms occurred near 0° C, confirming that water was the major frozen substance.

The results of fifteen rock coolings are summarized in Table 2. The water within rocks does undercool to some extent; a saturated rock has a higher freezing point (–3.8° C) than an unsaturated rock (–5.1° C). The latter can be considered a more typical value for freezing in the Antarctic cryptoendolithic habitat because of conductivity probes in the field indicates that liquid water can be present at temperatures as low as –10° C (unpublished).

Water content (% of rock weight)	Freezing point ± S.D. (°C)	N
0.3%	–5.15 ± 0.13	3
1.5%	–4.95 ± 0.70	5
saturated (approx. 1.9%)	–3.84 ± 1.06	7

Table 2: Freezing temperature of water in colonized Beacon sandstone at different water contents.

3.2 *High-frequency temperature oscillations*

Cultures of *Hemichloris antarctica* (7 and 42 days old) showed no significant change in reproductive viability after experiencing oscillations either between 5° C and –5° C at 1.5° C min^{-1} (held at –5° C for 0.5 min) or between 5° C and –10° C at 1.0° C min^{-1} (held at –10° C for 5 min), even after 50 cycles (Table 3). Transmission electron

microscopic study of *H. antarctica* and *Trebouxia* sp. did not find any evidence of ultrastructural damage after 50 cycles between 5° C and –5° C (unpublished).

Temperature range (cooling/warming rate)	Culture age(d)	Thermal cycles	Viability ± S.D. (percent)
5° C to –10° C	7	0	100± 32
(1.0° C min^{-1})	7	1	101 ± 43
	7	20	76 ± 30
	7	50	83 ± 42
5° C to –10° C	42	0	100 ± 46
(1.0° C min^{-1})	42	1	80 ± 27
	42	20	77 ± 24
	42	50	74 ± 33
5° C to –5°C	7	0	100 ± 14
(1.5° C min^{-1})	7	1	105 ± 39
	7	20	81 ± 11
	7	50	77 ± 43
5° C to –5° C	42	0	100 ± 35
(1.5° C min^{-1})	42	1	107 ± 23
	42	20	85 ± 35
	42	50	105 ± 58

Table 3: Reproductive viability of *Hemichloris antarctica* after cycles of cooling and warming.

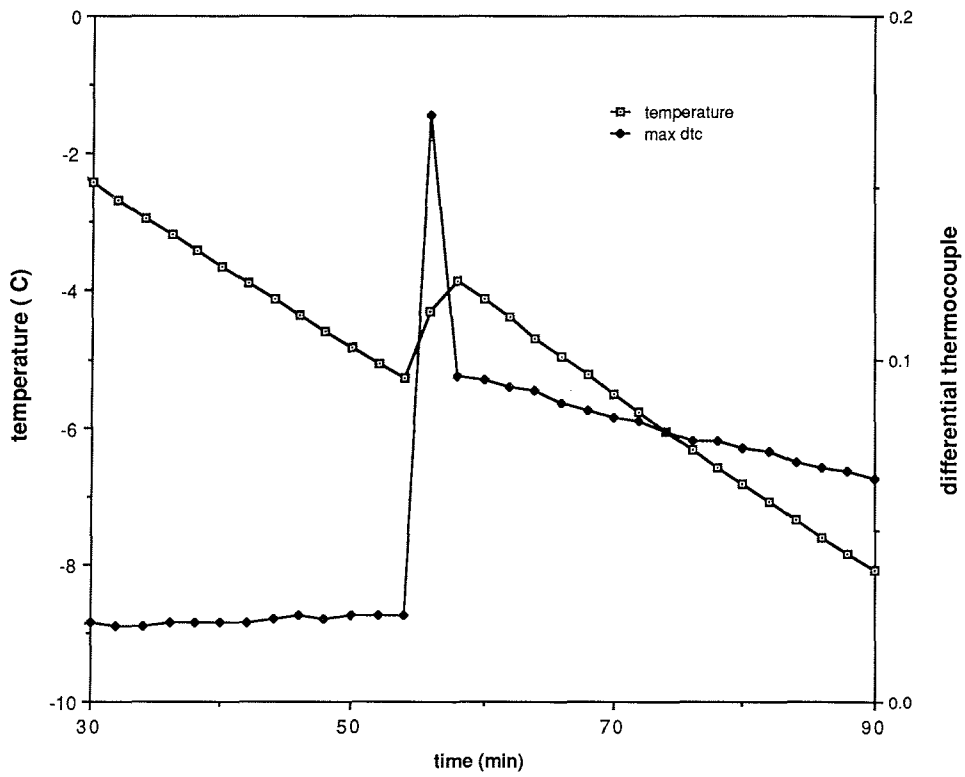

Fig. 1: Time course of temperature (mean) and differential-thermocouple output (maximum dtc) for each 2-minute interval as colonized Beacon sandstone cools through the freezing point.

3.3 *Freezing tolerance*

The cryomicrographs in Figure 2 show *Hemichloris antarctica* cells cooling 1° C min^{-1} from 5° C to –50° C and then warming to 5° C at 10° C min^{-1}. Prior to freezing, the cells were maintained at 5° C. At –2.5° C, the ice front had just passed the field of view, dislocating the cells but with no morphological changes evident. At –10° C, the

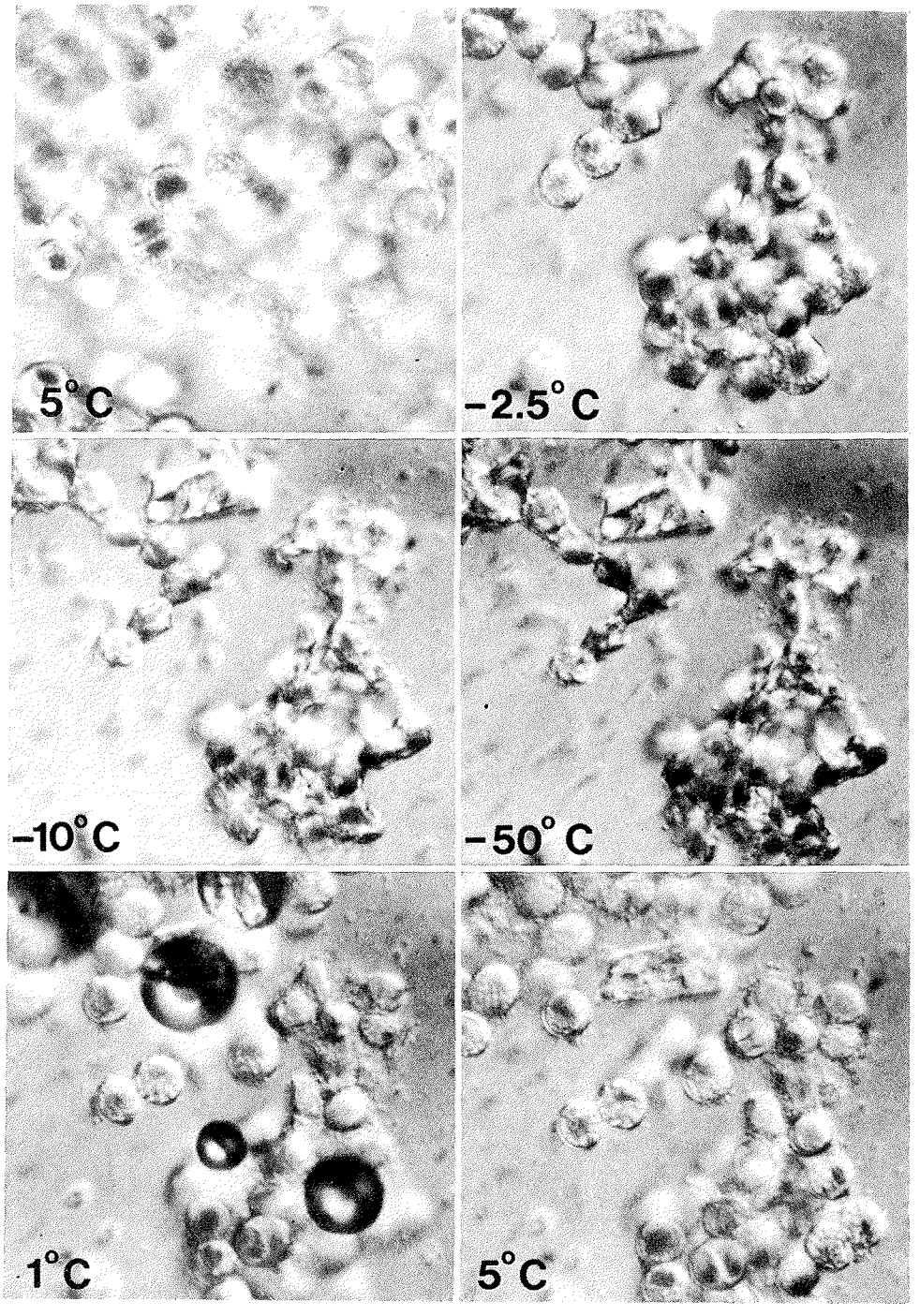

Fig. 2: Cryomicrographs of *Hemichloris antarctica* frozen at a rate of 1° C min^{-1} from 5° C to −50° C and thawed at 10° C min^{-1} to 5° C.

cells have noticeably shrunk, having lost water to extracellular ice, and the cell wall has collapsed along with the plasma membrane. At –50° C, there is little change in the cells except that slightly more crenation is evident. Upon thawing (1° C), the cells quickly re-hydrate and appear normal. Note that extracellular gases forced out of solution during freezing have now coalesced to form large bubbles. At 5° C, the cells appear indistinguishable from cells prior to freezing.

In a separate experiment, *H. antarctica* was rapidly frozen at 40° C min^{-1} to –50° C and then allowed to thaw at 10° C min^{-1} under the cryomicroscope. Although the cells had little time to lose water during freezing, intracellular ice apparently did not form, and no changes were evident.

Freezing tolerance was also tested by comparison of photosynthetic uptake of radiolabeled carbon (Table 4). Cells were cooled at 0.3° C min^{-1} to –20° C (freezing was initiated at –5° C). Upon reaching –20° C, cells were maintained at that temperature for 5 minutes and then warmed at 1° C min^{-1} to 10° C. The uptake of carbon by *Hemichloris antarctica* and *Trebouxia* was unaltered by freezing. When held at –20° C for 24 hours, *Hemichloris* was still unaffected by this freezing-thawing regime. However, there was a significant (t-test, alpha = 0.05) 7% decrease in carbon uptake by *Stichococcus* after 5 minutes at –20° C.

Organism (culture)	Period of freezing	^{14}C uptake ± S.D. (CPM)	N
Stichococcus sp.	control	59,345 ± 947	3
	5 min	55,051 ± 2460	3
Trebouxia sp.	control	15,226 ± 2545	4
	5 min	16,506 ± 1039	4
Hemichloris antarctica	control	73,350 ± 2242	4
(CCME 189)	5 min	77,743 ± 3195	4
	control	25,787 ± 1175	5
	24 hr	25,925 ± 763	5

Table 4: Effect of freezing to –20° C on $^{14}CO_2$ uptake by Antarctic algae.

4. DISCUSSION

In the cryptoendolithic environment, microorganisms are frozen for most of the year and are subjected to diurnal freeze-thaw cycles during the summer. We can assume, therefore, that the microorganisms have developed adaptations to such conditions. As expected, experiments demonstrated that cryptoendolithic algae can tolerate rapid cycling around 0° C, slow rates of freezing to –20° C, and freezing, even very rapidly, to –50° C. This degree of freezing tolerance has not been seen in other Antarctic free-living green algae (HOLM-HANSEN 1963) but has been demonstrated for lichen phycobionts (KAPPEN & LANGE 1970a, 1970b; LANGE & KAPPEN 1972). Among the algae studied, only *Stichococcus* showed some freezing damage, perhaps because this strain was isolated from a less severe environment than the endolithic algae.

Although endolithic algae are not injured by freezing, their metabolic rates may be reduced in a frozen environment. For example, several relevant physical changes occur during freezing:

— water activity decreases (at –5° C, the vapor pressure over ice is equivalent to 95% relative humidity over water)
— dissolved gases, such as CO_2, (cf. KAPPEN & FRIEDMANN 1983), may come out of solution
— a solid barrier to diffusion forms.

It is worth considering in this context that metabolic activity in the cryptoendolithic environment occurs mostly at sub-zero temperatures. Thus, in 1985—86, the total time for potential metabolic activity in horizontal rocks was 40 hours over 0° C and 180 hours between –5° C and 0° C (taken from Fig. 5, FRIEDMANN et al. 1987). Because the freezing point inside the rock is depressed to approximately –5° C, liquid water is available in this temperature range. This may not be the case on the rock surface, where freezing probably occurs at higher temperatures (the ice nuclei present in the environment will initiate ice formation). Therefore, even though cryptoendolithic algae are apparently well adapted to their freezing environment, the freezing regime of the rock surface may still be a contributing factor to its abiotic nature.

5. ACKNOWLEDGEMENTS

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References

Clarke, K. J. & Leeson, E. A. (1985): Plasmalemma structure in freezing tolerant unicellular algae. — Protoplasma 129: 120—126.

Franks, F. (1985): Biophysics and Biochemistry at Low Temperatures. — 210 pp., New York.

Friedmann, E. I., Hua, M. & Ocampo-Friedmann, R. (1988): Cryptoendolithic lichen and cyanobacterial communities of the Ross Desert, Antarctica. — Polarforschung 58: 00—00.

Friedmann, E. I., Friedmann, R. O. & McKay, C. P. (1981): Adaptations of cryptoendolithic lichens in the Antarctic Desert. — In: Jouventin, P., Massè, L. & Trèhen, P. (eds.) Colloque sur les Ecosystèmes Subantarctiques. Paimpont: 65—70, Paris.

Friedmann, E. I., McKay, C. P. & Nienow, J. A. (1987): The cryptoendolithic microbial environment in the Ross Desert of Antarctica: Satellite transmitted continuous nanoclimate data, 1984 to 1986. — Polar Biol. 7: 273—287.

Friedmann, I. (1966): Microscopy of algal chromatophores. — Phycologia 6: 29—36.

Greevey, J. P. & Whalley, W. B. (1982): The geomorphic significance of rock temperature variations in cold environments: A discussion. — Arctic and Alpine Res. 14: 157—162.

Guillard, R. R. L. (1973): Division rates. — In: Stein, J. R. (ed.) Handbook of Phycological Methods and Growth Measurements: 289—311, Cambridge.

Holm-Hansen, O. (1963): Viability of blue-green and green algae after freezing. — Physiol. Plant. 16: 530—540.

Kappen, L. & Friedmann, E. I. (1983): Ecophysiology of lichens in the dry valleys of Southern Victoria Land, Antarctica. II. CO_2 gas exchange in cryptoendolithic lichens. — Polar Biol. 1: 227—232.

Kappen, L. & Lange, O. L. (1970a): The cold resistance of phycobionts from macrolichens of various habitats. — Lichenologist 4: 289—293.

Kappen, L. & Lange, O. L. (1970b): Kälte resistenz von Flechten aus verschiedenen Klimagebieten. — Ber. Dtsch. Bot. Ges. N.F. 4: 61—65.

Lange, O. L. (1966): CO_2-Gaswechsel der Flechte *Cladonia alcicornis* nach langfristigem Aufenthalt bei tiefen Temperaturen. — Flora 156: 500—502.

Lange, O. L. & Kappen, L. (1972): Photosynthesis of lichens from Antarctica. — Antarct. Res. Ser. 20: 83—95.

McGrath, J. J. (1987): Temperature-controlled cryogenic light microscope — an introduction to cryomicroscopy. — In: Grout, B. W. W. & Morris, G. J. (eds.) The Effects of Low Temperatures on Biological Systems: 234—267, Baltimore.

McKay, C. P. & Friedmann, E. I. (1985): The cryptoendolithic microbial environment in the Antarctic cold desert: Temperature variations in nature. — Polar Biol. 4: 19—25.

Tschermak-Woess, E. & Friedmann, E. I. (1984): *Hemichloris antarctica*, gen. et sp. nov. (Chlorococcales, Chlorophyta), a cryptoendolithic alga from Antarctica. — Phycologia 23: 443—454.

Vestal, J. R. (1988): Carbon metabolism of the cryptoendolithic microbiota from the Antarctic desert. — Appl. Environm. Microbiol. 54: 960—965.