57th EASTERN SNOW CONFERENCE
Syracuse, New York, USA, 2000

Taxonomy and Biophysical Properties of Cryophilic Microalgae and Their Environmental Factors in Northwest Spitsbergen, Svalbard

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ABSTRACT

Snow fields and glaciers are habitats of microalgae that developed a physiology which is optimized to such an extremely cold and nutrient-poor environment. Five expeditions to Spitsbergen, Svalbard, in consecutive years (1995...1999) have provided an insight into the dispersal, distribution, and the environmental factors that affect snow algae. The last expedition laid the foundation for laboratory cultures enabling an update of an algal checklist. Our special interest is to understand the physiological processes that occur in a snow algal cell as it responds to temperature changes. We suspect that during this adaptation changes occur in the permittivity and conductivity of the cell membrane as well as of the cytoplasm, changes that are necessary to maintain transmembrane metabolism on an optimal level. Using the highly sensitive single cell spectroscopy (Gimsa et al. 1996), changes in both parameters can be measured. Currently we are conducting some preliminary work which is necessary for the adaptation of this method to work with snow algae. First results of this work and our hypothesis will be presented in this paper.

Key Words: Cryophilic algae, Single cell spectroscopy, Snow algae, Spitsbergen, Svalbard

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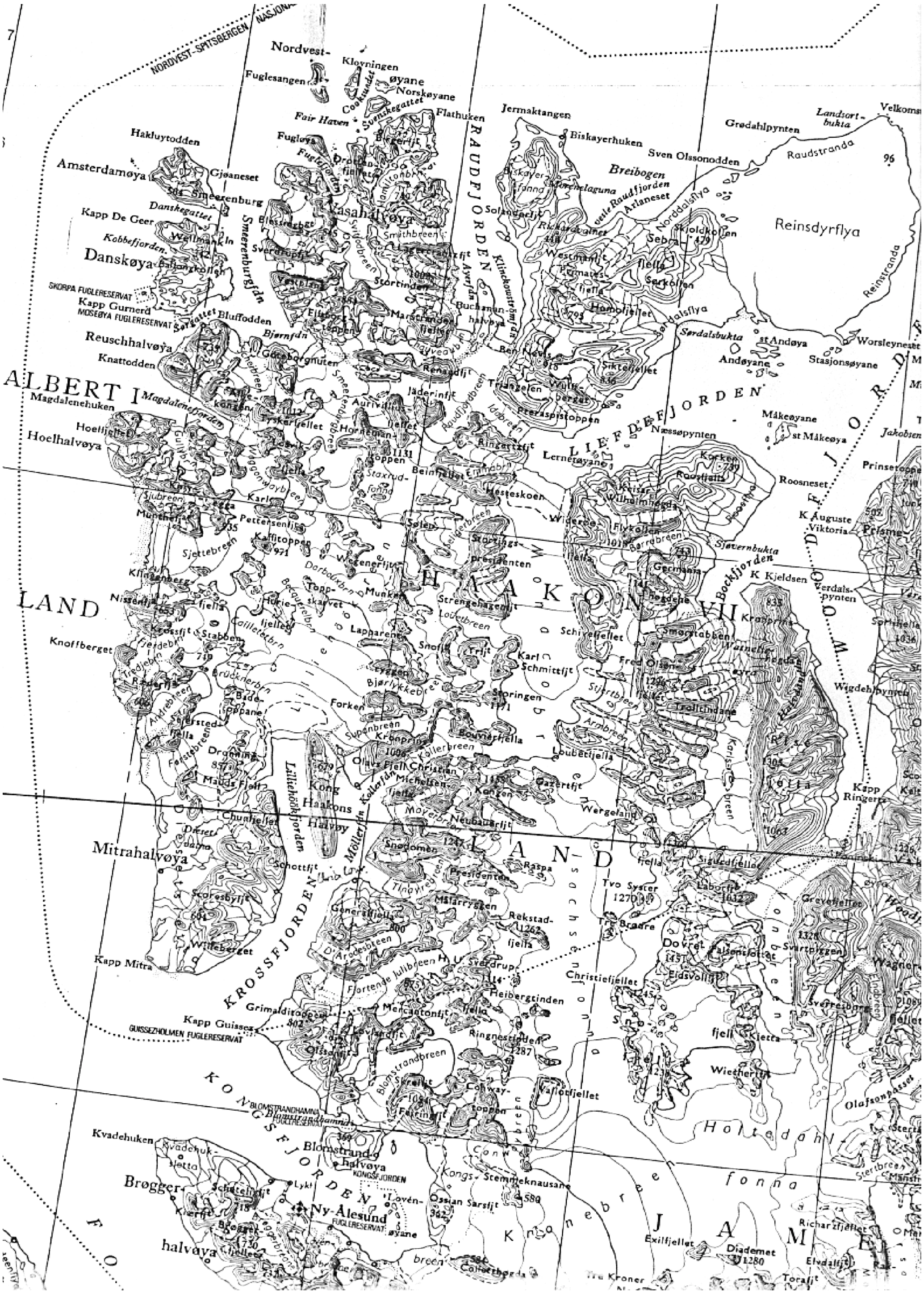

Figure 1. The northwest coast of Spitsbergen, Svalbard, showing eight major sampling sites of the 1999 expedition KOL 07.

THE RESEARCH AREA

Spitsbergen is the largest island of the Svalbard archipelago located in the Arctic Ocean approximately 1000 km north of Norway and 900 km south of the North Pole. Large areas of Spitsbergen are covered with glaciers and persistent snow fields which often extend right down to sea level, facilitating access and research considerably. Because of the northernmost extensions of the North-Atlantic Drift (Gulf Stream) along the west coast of Spitsbergen this area has a moderate cool summer climate with day temperatures around +5 °C and rarely rising above +10 °C. The mean maximum temperature between June and September 1999 was +5.3 °C (http://www.awi-bremerhaven.de/MET/NyAlesund/obsequery2.html). However, in midwinter (January and February), minimum temperatures may drop below –25 °C. Most research on Svalbard is conducted in Ny-Ålesund (N 78.5° E 12°), covering marine and terrestrial biology, climate research in the troposphere and stratosphere, space geodesy, and air quality research and arctic pollution.

SNOW ALGAE

Our 1999 expedition (26 August to 16 September) led us along the northwest coast to eight major sampling areas (Blomstrand, Stuphallet, Vestre Brøggerbreen, Kong Haakons, Hamburgbukta, Bjørnhamna, Amsterdamøya, Makarovbreen) (Fig. 1), each with several different sublocations with regard to snow quality and persistency, orientation, slope, etc. With some exceptions, green and red snow fields were observed regularly from ship along the coastline and further inland. The majority of the snow algal fields showed different shades of red. Several snow samples were taken from each location and studied microscopically on board for algal species and culture isolations. Live samples were also carried home for further studies and cultures.

Algae and cyanobacteria representing seven classes were found with the Chlorophyceae clearly dominating (see Table 1).

Table 1. Taxa list of cryophilic and soil algae and fungi found during KOL 7 expeditions (until Sept. 1999) on persistent and nonpersistent snow fields in North-West Spitsbergen, Svalbard. (index CCryoHUB = strain held at the Culture Collection of Cryophilic Algae at the Humboldt-University Berlin).

Cyanophyceae
Nostoc sp.-1
Oscillatoria sp.
Oscillatoria sp.-thick-thread CCryoHUB
Phormidium sp.

Chlorophyceae
Chlamydomonas nivalis (Bauer) Wille 1903
Chlamydomonas sp.
Chlamydomonas sp.-bell
Chlamydomonas sp.-cup-chlp
Chlamydomonas sp.-egg
Chlamydomonas sp.-elongate-swarmer
Chlamydomonas sp.-hyaline-layer
Chlamydomonas sp.-long-narrow
Chlamydomonas sp.-red-oval
Chlamydomonas sp.-stigma-lateral CCryoHUB
Chlamydomonas sp.-violet-red-sediment
Chlamydomonas sp.-wide-shoulder
Chlorella sp.
Chlorella sp.-glitter
Chlorococcum sp.
Chlorococcum sp.-goldgreen
Chloromonas brevispina (Fritsch) Hoham, Roemer et Mullet comb. nov. 1979
Chloromonas nivalis (Chodat) Hoham &

Pleurastrophyceae
Myrmecia biatorellae (Tschermak-Woess & Plessl) J.B. Petersen 1956 CCryoHUB
cf. *Trebouxia* sp.-orange-plaques CCryoHUB

Zygnematophyceae
Ancylonema nordenskiöldii Berggren 1871
Cosmarium debaryi W. Archer 1861 CCryoHUB
Cosmarium cf. *goniostichum* Skuja 1964
Cosmarium cf. *hammeri* var. *homalodermum* (Nordstedt) West CCryoHUB
Cosmarium holmiense P. Lundell 1871 CCryoHUB
Cosmarium ralfsii Brébisson ex Ralfs 1848 CCryoHUB
Mesotaenium berggrenii (Wittrock) Lagerheim (1892)

Charophyceae
Klebsormidium flaccidum (Kützing) Silva, Mattox & Blackwell 1972 CCryoHUB
Klebsormidium cf. *scopulinum* (Hazen) nov. comb.
Raphidonema berninum Kol 1935
Raphidonema nivale Lagerheim CCryoHUB
Raphidonema tatrae (Kol in Györffy) Kol in Vischer 1933
Stichococcus bacillaris Nägeli 1849 s.l. CCryoHUB
Stichococcus sp.-1

Mullet comb. nov. 1978 [CCryoHUB]
Chloromonas sp.-chloro-disc [CCryoHUB]
Chloromonas sp.-granular-longflagellae [CCryoHUB]
Chloromonas sp.-green-glitter
Chloromonas sp.-green-to-end
cf. *Chloromonas* sp.-little-orange-spores
Chloromonas sp.-truncated [CCryoHUB]
Chloromonas sp.sticky-flagella
cf. *Coenochloris* sp.-1
Cystomonas sp.
cf. *Desmococcus* sp.-packages
cf. *Desmococcus* sp.-trichal [CCryoHUB]
Hazenia mirabilis Bold 1958
Macrochloris sp.-1
cf. *Rhexinema* sp.
Tetracystis sp.-1 [CCryoHUB]
Tetracystis sp.-orange-lobe [CCryoHUB]
cf. *Tetraspora* sp.-1
Stichococcus cf. *undulatus* Vinatzer 1975 [CCryoHUB]

Bacillariophyceae
Gyrosigma sp.
Melosira sp.-chlp-ribbonlike
Melosira sp.-lentils
Nitzschia sp.-blunt-ends
Nitzschia sp.-button-ends
Pinnularia sp.-oil-droplets

Dinophyceae
Gymnodinium sp.-yellow-green

Fungi
Chionaster nivalis (Bohlin) Wille 1903
Chytridiomycetes
Selenotila nivalis Lagerheim [CCryoHUB]

Approximately 63 taxa from 28 genera were identified, with a predominance of *Chlamydomonas* and *Chloromonas* species. Also three taxa of fungi, among them a parasitic Chytridiomycete, were found. In our Culture Collection of Cryophilic Algae at the Humboldt-University Berlin (CCryoHUB) we currently hold 19 taxa of identified strains plus several yet unidentified clones, most of them in axenic agar cultures kept at +1 °C and 16:9 (L:D) light cycle. Field findings, taxa information, photomicrographs, and strains kept in the collection are organized in a Microsoft Access database. Depending on the nature of the location, well-established soil algae (e.g., *Chlorococcum*, *Macrochloris*, *Tetracystis*, and *Desmococcus*) intermingle with typical snow algae; however, they may not be considered as cryophilic. Same applies to most of the Cyanophyceae, Bacillariophyeae, and *Cosmarium* species. For definite identification of many taxa, genetic sequencing of the 18s rDNA (SSU rDNA) genome will be conducted, also to compare morphologically different taxa from different locations (within our research area, but also comparing Arctic and Antarctic taxa). A first sequence revealed a 98% identity of our taxon "CCryoHUB 002b-99 – *Chlamydomonas* sp.-stigma-lateral" with an unidentified strain "Antarctic 2E9" collected by a Japanese research group from Antarctica (personal communication). Using the same methodology to sequence *Chlamydomonas reinhardtii* resulted in a 99% identity with data base results. We will continue to use 18s rDNA sequencing as a tool to identify and compare our strains.

TEMPERATURE AND CONDUCTIVITY OF SNOW FIELDS

From July 1998 until August 1999 an automatic temperature data logger was deposited at a site on Blomstrandhalvøya (N 78°59' E 12°07'). Temperature data were stored every six hours. They showed varying top soil temperatures between +20 °C and –17 °C from mid-July until mid-December 1998. From the beginning of 1999 a persistent snow cover stabilized the temperature at –7.2 °C until May 1999. From then on, with rising air temperatures, the snow cover began to melt and the wet soil with melted snow and ice showed a stable temperature of –0.6 °C for the month of June and half of July. As summer progressed, the soil surface became drier and was fully exposed with temperatures occasionally rising up to +22.0 °C, but usually ranging between +3 and +10 °C, until temperatures again began to fall at the end of summer. From this temperature profile it may be concluded that in winter snow algae covered by snow are not exposed to unstable conditions of –20 to –30 °C but experience a reasonably high and constant temperature of around –7 °C.

At various sampling locations the pH value and conductivity (σ) of snow on the algal fields were measured. Generally, the pH value ranged between 5.0 and 5.4 and conductivity values were low, between 12 and 60 µS cm^{-1}. Only where snow fields near sea level were influenced by sea

spray (e.g., Kong Haakons) were both pH and σ values slightly higher (6.3 and 100 µS cm^{-1}, respectively). Conductivity values of around 100 µS cm^{-1} were also observed in the middle of the Makarovbreen (glacier), far away from influence of bird colonies. We suspect that the elevated values result from the special character of the ice in these areas, which is of an icy and granular structure. Ions from mineral sources from the surrounding rocks may be trapped on these non-melting snow/ice areas and accumulate. Interestingly these isolated glacier surfaces proved to be the typical habitat for the characteristic snow algae *Ancylonema nordenskiöldii* and *Mesotaenium bergrenii*.

SINGLE CELL SPECTROSCOPY

This method is a highly sensitive, yet non-invasive method to characterize the passive electrical properties of individual cells (Arnold and Zimmermann 1988; Fuhr et al. 1990; Fuhr et al. 1996; Gimsa et al. 1996). The rotation of cells and microscopically small particles induced by a rotating electrical high frequency field is the basis of this method. The rotational effect is due to interactions of the electrical field with charges induced at the interfaces of different cell compartments. The rotational behavior of a cell, the rotation spectrum, reflects the dielectric properties (permittivity ε, conductivity σ) of these single cell compartments, especially of the cell wall, cell membrane and the cytoplasm. The frequency-dependent dielectric differences between particles and surrounding medium result in an induced translational force (*F*) and a torque (*N*) which are interrelated and result in a motion known as electrorotation (ER) and as dielectrophoresis (DEP) in an inhomogeneous field. In general, each relaxation process (e.g., at the interface of single cell compartments or due to dispersions of single rotation spectra of several cell compartments) can yield one electrorotational peak in the rotation spectrum. When graphically analyzing such a spectrum, a clockwise direction of the rotation is considered as a negative velocity, whilst anticlockwise rotation is regarded as positive. The theory of electric field mediated force calculation is summarized extensively by Jones (1995).

In our case, with freshwater algae, the effective conductivity of the outer medium is generally lower than that of the cell's interior. Thus, at frequencies up to 250 MHz positive dielectrophoresis occurs—cells are being attracted to the electrodes. To avoid this and also to suppress dragforce of the cell on the substrate, the algal cells are held contactless by infrared laser tweezers approximately 40 µm away from the electrodes. A MOSPAD (micro-optical single particle detector) system automatically measures the rotation velocity and to control the temperature the micro electrode chip with the algal cell to be measured is cooled with N_2-cooled air.

Having the fluid mosaic model of membranes in mind, a membrane remains fluid at a lower temperature if it is rich in phospholipids with unsaturated hydrocarbon tails. And this fluidity is necessary for the membrane to stay biologically active. From algae grown in laboratory cultures and exposed to lowered growth temperatures it is a known response to increase the proportion of polyunsaturated fatty acids in the polar lipids of biomembranes (Henderson and Mackinlay 1989). Bidigare et al. (1993) found elevated proportions of monounsaturated fatty acids in red resting stages of *Chlamydomonas* spp. from Antarctica; however, in the green vegetative stage of these cryophilic algae, the proportion of saturated fatty acids was predominant. Morris et al. (1981) observed that cells of *Chlamydomonas reinhardtii*, a non-cryophilic species, which they adapted to cold growth temperatures, even decreased their contents of unsaturated fatty acids. This contradictive observation, however, may be due to the fact that *C. reinhardtii* is not a cryophilic algae.

Cells adapting to cold temperatures have to take care of three processes: (1) changing the ratio of saturated and unsaturated fatty acids in the lipids of the cell membranes to maintain membrane fluidity (Morris et al. 1981; Roessler 1990), (2) adjusting the permeability of the cell membrane for water to react to osmotic changes of the outer medium (Kawecka 1986), and, (3) when exposed to freezing temperatures reducing the water content of the cell interior to prevent ice crystal formation (Giese 1973). We are confident that with single cell spectroscopy effects of these three

reactions can be visualized, especially when the temperature rises above the viability threshold. The last point is the main reason why we are interested in using temperature sensitive snow algae.

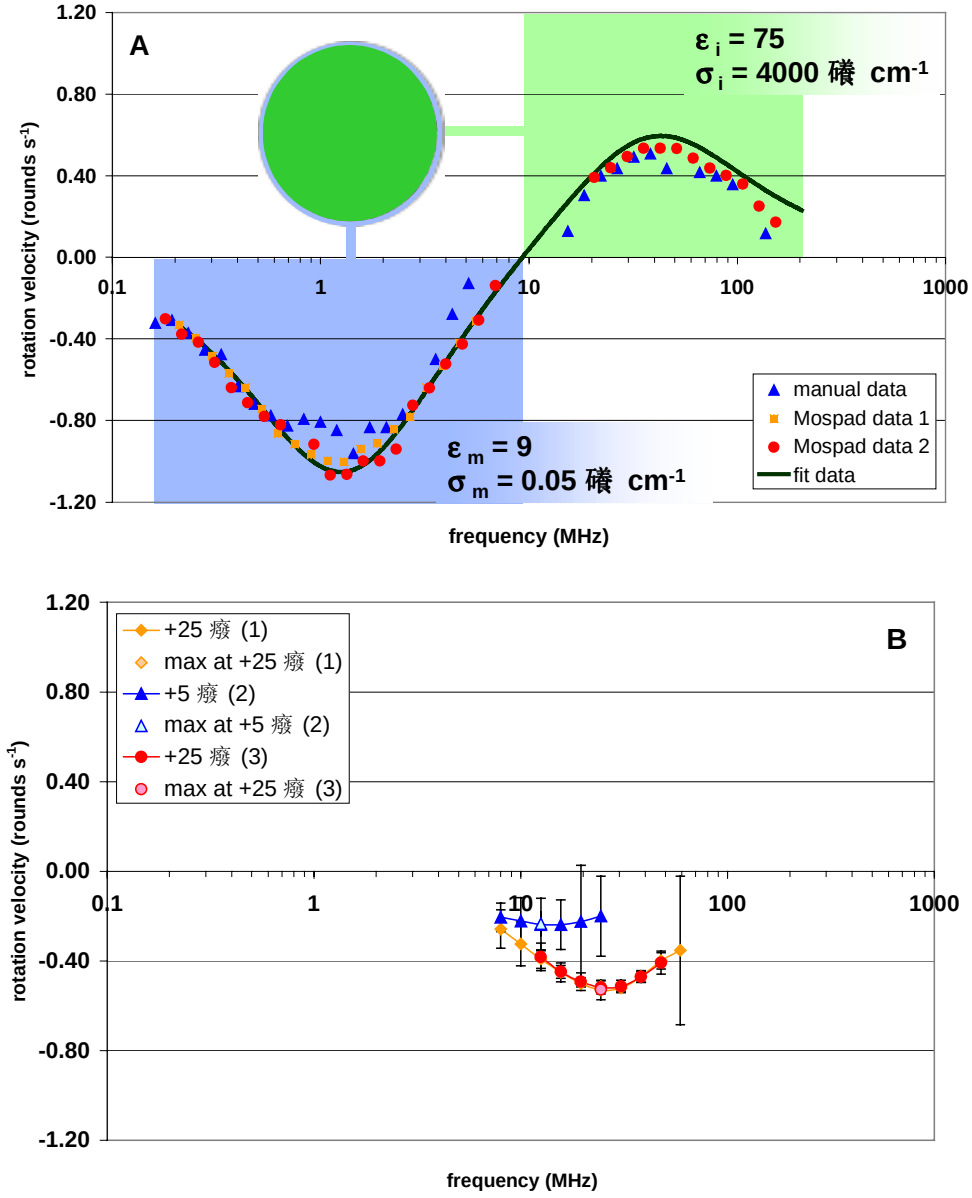

Figure 2. (A) Single cell rotation spectra of a coccal algal cell and the mathematical fit to calculate conductivity (σ) and permittivity (ε) of the cell membrane (index $_m$) and cytoplasm (index $_i$) ◢ = velocity data measured with a stop watch, ● / ■ = velocity data registered twice with the MOSPAD system, — = mathematical fit of the MOSPAD data according to equations described by Gimsa et al. (1998). (B) Dependency of the rotation velocity on the temperature when measuring a polystyrene bead as a model system. First measurement (◆) was taken at +25 °C, second (◢) after cooling down the same bead to +5 °C and the third (●) again after warming the same bead to +25 °C. Deviation bars are standard errors with n = 10. Open symbols mark the maximum velocity.

We have conducted first electrorotation experiments (Fig. 2) and when analyzing rotation spectra mathematically, the values for conductivity and permittivity of the cell membrane (first, negative peak) and of the cell interior (second, positive peak) can be calculated (see Fig. 2A). Next step is to measure the same cell at a different temperature. However, one has to take into account that with changing temperature also the viscosity of the outer medium and its conductivity are

changing. Lower temperatures result in a higher viscosity which causes a decrease in the rotation velocity (see Fig. 2B). These parameters have a considerable influence on the rotation spectrum and may superpose changes that are happening on membrane or cytoplasm level to maintain membrane fluidity as a response to the temperature change. With preliminary experiments we are investigating this problem now to be able to calculate it when applying the mathematical fit to further rotation data obtained later.

We suspect, however, that we finally will be able to detect changes in conductivity and permittivity in the cell wall and cytoplasm respectively, which can be regarded as a response to changing temperatures of the outer medium.

ACKNOWLEDGMENTS

We thank our colleagues for their assistance: C. Reichle at the laser tweezers and MOSPAD, Dr. Th. Schnelle for mathematical modeling of rotation spectra, and Dr. A. Farouk and Prof. Dr. R. Borriss for introduction to genetic sequencing. We are grateful to the Deutsche Forschungsmeinschaft (DFG) for funding the long-time project KOL 7 (fund no. Fu 345/6-2) in cooperation with the Alfred Wegener Institute for Polar and Marine Research.

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