Survival and Living in Ice

Cultivation-Independent Identification and Phylogenetic Relations of Microorganisms from Young Glacier Ice

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Introduction

Cold-extreme habitats are common:

Polar regions account for more than 14% of the earth's surface.

90% of the oceans are colder than 5 °C

→ How significant is the biosphere in these environments?

Life in ice poses a number of challenges to organisms:

•Biochemical reaction rates are slow • Nutrients for growth and chemical energy sources are scarce • Liquid water is lacking most of the time

Microorganisms adapt to living at low temperatures employing a number of different strategies: Spore formation, converting into resting, non-growing stages, production of extracellular mucilage which induces freezing around but not inside cells, increased intracellular solute concentration to prevent freezing damages, changes in the lipid and protein composition of membranes to make them more elastic.

Organisms living in the cold offer insight into new life strategies and they might lead to the discovery of new biotech-products.

→ Which organisms can be found in young glacier ice? Do relations to cold-dwelling microbes from polar regions exist?

Sampling

Jungfraujoch (CH), 3450m above sea level / Nov. 6. 2003

Samples: Ice cores (1 m long, diameter 90 mm) from granular ice-layers below snow cover of approx. 1.3m

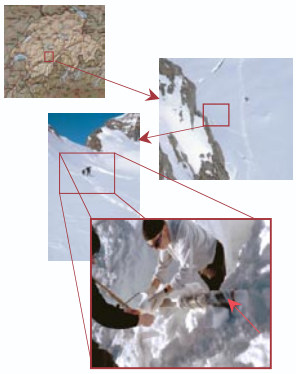

Fig.1: Samples were obtained by drilling cores (1m length, Ø 90 mm; red arrow) into granular ice layers of Jungfraufirn glacier near the JFJ research station. The samples were packed into sterile, water tight bags

Methods

Samples

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DNA-Extraction (Durapore ® Membrane filters)

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PCR with different primers for rRNA-genes

Primer pairs for 16s/18s rRNA genes

Universal	uni 519s – uni 1392as
Bacterial	bact 0008s – bact 1524as
Archeal	Arch 89F – Arch 915R
Eukaryotic	arch 0008s – 1512uR

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Insertion of PCR-products into Plasmids

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Insertion of Plasmids into *E.coli*

cloning

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Growth of *E.coli* and selection of single clone colonies

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PCR with M13-primers to get inserted rRNA-genes → Check: RFLP (restriction fragment length polymorphism); Digestion of rRNA-DNA with restriction enzymes HaeIII and HinfI

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Sequencing

Results

a) PCR:

-**universal and bacterial** primers resulted in amplification of rRNA-genes

-**no archean** live forms could be detected

b) RFLP-patterns:

Digestion of rRNA-genes (HaeIII and HinfI). The resulting RFLP-patterns indicate, that approx. 26 different rRNA-genes could be expected after sequencing (red arrows in figure below).

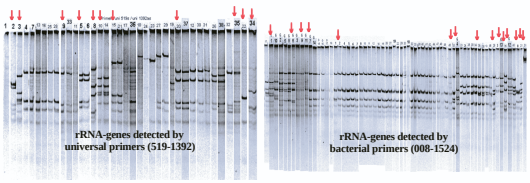

Fig. 3: The different patterns on the polyacrylamid-gels result from sequence-specific digestion of rRNA-genes employing HaeIII and HinfI. Identical or very similar sequences show the same patterns. Red arrows indicate the clones chosen for sequencing.

c) Analysis of sequenced rRNA-genes

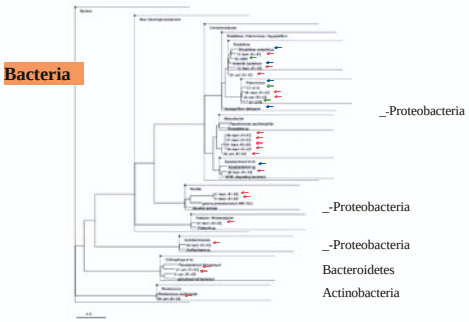

Fig. 4: 17 different sequences of bacterial 16S rRNA-genes were found (red arrows). Several species are closely related to psychrophilic organisms found in polar regions (blue arrows)[1]. Interestingly, also relations to bacteria found in the cold freshwater habitat of Jöri Lake XIII, are present in the Jungfraujoch ice (green arrows)[2].

Family Comamonadaceae

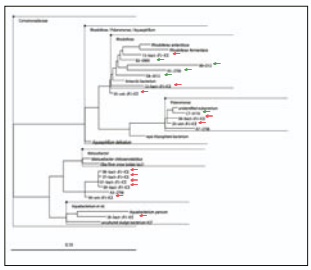

Fig. 5: Comamonadaceae: more than half of the sequences found belong to species from this family. Many organisms of this group were also found in cold habitats such as sea ice of the Arctic/Antarctic.[1]

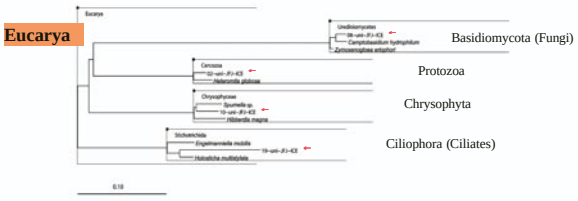

Fig. 6: Eukaryotic sequences found in young glacier ice; in sea ice, eukaryotic algae and protozoa play an important ecological role in primary production. The role of the eukarya found in young glacier ice samples from JFJ needs still to be examined.

Conclusions

A high diversity of microorganisms can be found under the extreme conditions in glacial ice.

Many of the sequenced rRNA-genes indicate the presence of species that are closely related to already known psychrophilic (cold-loving) organisms.

References:

[1]James T. Staley, John J. Gosink, "Poles Apart: Biodiversity and Biogeography of Sea Ice Bacteria"; Annu. Rev. Microbiol. 1999. 53: 189-215

[2]see poster O11/O12 M. Yuhana, G. Iqbal-Nava, K. Hanselmann