DANMARKS OG GRØNLANDS GEOLOGISKE UNDERSØGELSE RAPPORT 2003/22

Water quality of the Greenland icecap

Mikkel Bender, Gitte Felding and Carsten Suhr Jacobsen

GEOLOGICAL SURVEY OF DENMARK AND GREENLAND
MINISTRY OF THE ENVIRONMENT

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Summary:

The projekt ”Drikkevandskvalitet af Grønlandsk indlandsis” is a research project financed by Greenland Resources A/S. The project aim has been to investigate the possible chemical and microbiological contamination in ice from the Greenland icecap, in order to asses the potential use of this resource in the production of water or ice for human consumption.
The project was initiated as part of a tandem project to the project “Global forurening af den grønlandske indlandsis med miljøfremmede stoffer og tilstedeværelse af levedygtige mikrobielle kim” financed by DANCEA under the Danish Ministry of the Environment. While the DANCEA-financed project was focused on the effects of globally transmitted pollution on the Greenland icecap, the present project has specifically aimed at assessing the quality of the ice as water for human consumption.

The project has primarily analysed ice from the glacier 1AH0200. After initiation of the analysis, potential problems were reported from Greenland Resources as visible silt in the large intact sample was reported from RISØ. However, GEUS consider this contamination to be of older date, and that the present sample is representative of the glacier. A new sample was received by GEUS in the fall of 2002. The present report, however, is based on the original sample.

Samples were taken aseptically and analysed for the content of Pesticides, PAH-compounds (Polycyclic Aromatic Hydrocarbons), PCB (Poly-Chlorated Biphenyl´s) and living microorganisms.

The ice from the glacier 1AH0200 (near Narssasuaq) did not contain any of the 72 pesticides, 15 PAH´s and 7 PCB´s analysed for above the detection limit. No chemical contamination in the ice, which is more than 1000 years old, was detected.

The microbial analysis showed fewer Colony Forming Units (CFU), than allowed as limiting values in Danish tap water. Numbers of colony forming units were strongly dependent on the cultivation media, and incubation temperature used. In general, numbers of isolated microorganisms were higher using nutrient-poor media and lower incubation temperatures. As expected, melting and storage of the melted ice under aerobic conditions resulted in microbial

growth. Numbers of colony forming units, however, reached a stable and relatively low level, due to the absence of nutrients (below detection limits).

Characterisation of re-grown microbial isolates using cultivation dependent techniques showed no micro-organisms closely related to known human pathogens. Based on sequencing the 16S rDNA genes, no isolates were shown to be closely related to known human pathogens. Only a small number of isolates, however, were characterised using molecular techniques.

Project occurrences:

The project was contracted and funded in August 2001. In September 2001 two GEUS-employees (Gitte Felding (chemist) and Ole Olesen (glaciologist)), travelled to Greenland. Assisted by people from Greenland Resources samples were collected from a glacier. The ice was shipped to GEUS in Denmark and was here analysed for presence of selected chemical compounds and microbial life.

Chemical data as well as data from total microbial colony forming units (CFU) and deposition data were completed in may 2002. Final data from the microbiological risk assessment, however, awaited identification of the microbial isolates from the ice. This work proved difficult and time-consuming as the bacterial isolates were difficult to re-culture and grew at very low rates. Every necessary culture step resulted in time-consuming periods of incubation. Finally a characterisation based on molecular sequencing of 16S ribosomal DNA sequences has been initiated. However, prior to receiving the results of these investigations it has been possible to show that the presence of pathogenic micro-organisms in the ice samples is unlikely.

Background:

The Greenland icecap constitutes a potential resource for export of drinking water. A description of the extent and accessibility of the glaciers of Greenland is to be found in GEUS-report 2000/13. The present report gives a chemical and microbiological risk assessment of ice from the icecap used as drinking water.

Chemical pollution enters the atmosphere primarily in the industrialised world. Though the arctic constitute a pristine and relatively untouched environment, unwanted compounds can potentially enter the arctic through precipitation.

Pesticides compromise a group of compounds used to optimise crop yields in agriculture. The extensive use of pesticides was initiated after the Second World War, and the present day use of pesticides is an estimated 300.000 tons active compound per year in Europe.

It has been estimated that the world-wide production of PCB´s (PolyChlorinated Biphenyl´s) has exceeded 1.5 million tons, peaking in the year 1970. PCB´s are very stable compounds. The water solubility of PCB´s is low, and potential presence in the ice will be adsorbed to particles encapsulated by the ice.

The AMAP assessment report (Arctic pollution issues. Arctic Monitoring and Assessment Program) from 1998 describes the presence of POP´s (Persistent Organic Pollutants) in the arctic environment. Primarily described compounds are HCH´s, PCB´s, DDT´s, PAH´s, HCB and toxaphenes.

The presence of these compounds in animals, sediment, sea water, lake water, air, rain and snow is determined. However, investigations on the presence of hazardous substances in the arctic environment are very limited. In sea, ice and snow from the Russian part of Kara and the Lartev Sea, presence of HCH, DDE, DDD, DDT and PCB has been shown. Air and snow from Canada, Norway and Russia has been examined for presence of POP´s (Kallenborn et al., 1991; Enge et al., 1991), however similar analysis from Greenland and Alaska are missing. The Greenland Icecap has been analysed for lead and mercury as well as the ions; Chloride, nitrate, sulphate, ammonia and hydrogen.

Organic Chlorinated pesticides (HCH, DDE, DDT) have been found in an alpine glacier, where the ice was estimated to have been formed in the period 1981-1988 (Villa et al., 2000). One survey of PAH´s in the Greenland icecap has been published (Masclet et al., 2000). Samples were taken at a depth of 5 metre and the ice represents the period 1989-1993. The ice was analysed fore 14 different PAH´s, originally formed by the burning of fossil fuel, exhaustion from cars, wood fires etc. The total concentrations given as the sum of 12 PAH´s (the exceptions were Phenanthrene and Naphthalene) were from 0.1 to 10 µg/kg. The pollution was assumed formed in Eastern Europe, Russia and North America.

In Denmark, bottled mineral water is allowed to contain the same amount of pesticides (0.1 µg/l of a single compound, 0.5 µg/l total) as normal tap water. The same criteria will apply to melted ice from the Greenland icecap. However a major sales argument could well be the expected extreme purity of the water from this source.

Directives from The Danish Ministry of Environment on microbiological quality of drinking water is given in the "directive for drinking water", which apply to Danish tap water. A maximum of 20 colony forming units (CFU´s)/ml incubated at 37°C and 200 CFU´s/ml incubated at 22°C is allowed, however guiding values are four times lower, 5 and 50 CFU/ml, respectively. Further, presence of coliform bacteria, thermotolerant coliform bacteria, faecal streptoccoci or sulphate-reducing *Chlostridium perfringens* is not allowed.

Directive 67 of 30. January 1998 from the Danish Ministry of Food, Agriculture and Fisheries states that the microbiological quality demands given in the "directive for drinking water" apply to natural mineral water and natural pond water measured at the source. Melted ice from the Greenland icecap can not directly be defined as natural mineral water but may be defined as natural pond water as above mentioned directive describes natural mineral water as "water, especially approved as such by the rules of the directive. The water must be derived from an underground source, protected by any risk of contamination and therefore naturally purified. Natural mineral water must in addition derive from a source where the texture and content does not change in time and space". In comparison it is stated on natural pond water: "Natural pond water is not approved separately, but has to meet some of the criteria applied to natural mineral water."

In a previous investigation we have found viable bacteria in a block of ice derived from an iceberg (Jacobsen, 2000). However, the sample was simply pulled out of the sea and due to potential contamination of seawater introduced by the sampling procedure, it is uncertain

whether the number of bacteria found represents the true number of bacteria present in the icecap from where the iceberg was derived.

Several publications in the international literature have shown that viable bacteria can be found in glaciers comparable to the Greenland glaciers. One publication compares results from glaciers found in different parts of the world (Christner et al., 2000). In a single analysis of two samples from the Greenland icecap they only found viable bacteria in one sample and in this case less than 1 CFU/ml.

From other parts of the world however it has been possible to isolate viable bacteria encapsulated in ice derived from a series of arctic and Antarctic habitats (Christner et al., 2000; Skidmore et al., 2000; Gordon et al., 2000). Investigations on ice of several hundred years of age derived from glaciers from Antarctica, Himalayas and Andes have shown viable bacteria in numbers up to 180 CFU/ml. In general, these viable bacteria belong to groups of bacteria capable of forming spores.

Analysis of glacier-ice from the Arctic Canada has shown that the ice contains a divers culturable flora of coliform bacteria. This culturability was however greatly inhibited by cultivation at 37°C. The investigators report their observation of several species of mammals on the glacier and the presence of coliforms in the ice could well be linked to these animals (Skidmore et al, 2000).

Besides aerobe bacteria other viable and culturable bacteria found in Canadian Glacier-ice include nitrate-reducing bacteria, sulphate-reducing bacteria and methanogenic bacteria. The numbers of viable bacteria is greatly dependent on the organic content in the ice as well as the composition of the cultivation media and the incubation temperature (Skidmore et al, 2000).

Bacteria adapted to life in cold environments are believed to show optimal growth at lower temperatures. Additionally bacteria of potential risk to humans are normally capable of growing at higher temperatures. This means that isolation of micro-organisms and bacteriological risk assessment of ice from the Greenland icecap must include isolation and re-isolation of bacteria at both high and low temperatures.

Environmentally hazardous compounds are, as described above, shown to be present in the arctic region. Data from the Greenland icecap, however, has so far not included compounds other than the PAH´s. Concordantly, only inconclusive and sporadic reports on the presence

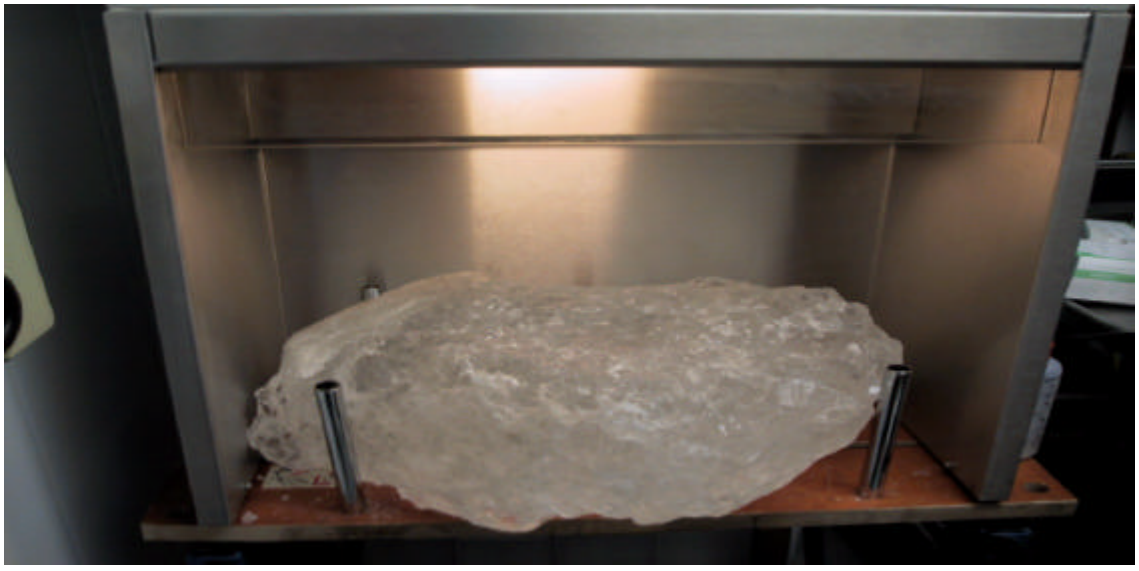
of viable bacteria in the Greenland icecap exist. This report will be the first attempting to describe the bacteria present in the Greenland icecap as well as describing which environmentally hazardous compounds has reached the arctic environment and been encapsulated in the icecap.

Sampling procedures:

The age of the ice from the icecap varies and it is therefore possible to describe global and local contamination events through time by analysing the ice. In September 2001 samples were collected from 4 locations. Prior to sampling the upper centimetres of the snow/ice/firn was removed to minimise possible contamination. The samples were stored in Rilsan-bags™, which do not leak any form of compounds to the sample. The temperature at the time of sampling was around 0°C and the samples were therefore stored in insulation-boxes until they arrived at the freezer-facility, where they were packed and shipped to Denmark as frozen cargo. The transfer was made by air or sea, in both cases subzero containment of the samples was ensured. After arrival to GEUS samples were kept at -20°C until the initiation of analysis.

The sample from glacier 1AH0200 (the primary interest of Greenland Resources) is in the following and in the appendix numbered as sample 3. We have in comparison included our results from the other samples analysed in connection with the project financed by DANCEA under the Danish Ministry of the Environment.

A frozen block of ice was cut in a freezer. In order to ensure aseptic handling of samples the work was done in a laminar flow bench and all tools used were sterilised prior to use. Sterile control samples were placed in the flow bench to detect possible contamination occurring during the handling of samples.



The outer surfaces of the block were melted aseptically in large sterile containers and sent to chemical analysis at the commercial laboratory Eurofins (formerly Miljø-kemi)."

From Narsarsuaq 3 samples were collected, 2 very young samples and 1 sample of ice estimated to be at least 1000 years old. This sample was collected approximately 200 meters above sea level on the glacier Qaleralitt Sermia. The 2 other samples were collected near by, approximately 1200 metres above sea level both representing the years 2000 and 2001.

Data and comments from analysed samples.

Sample number and sampling date	Location	Estimated age in years	Co-ordinates	Estimated height above sea level in metres	Comments
1 2-9-2001	Sdr. Strømfjord	>10000	67° 8`N; 60° 7`W	400	Poss. Contamination
2 9-9-2001	Narsarsuaq	1	61° 18,92`N; 46° 35,15`W	1250	Station 72
3 9-9-2001	Narsarsuaq	>1000	61° 00,00`N; 46° 40,83`W	200	Qaleralitt Sermia
4 9-9-2001	Narsarsuaq	1	61° 16,44`N; 46° 47,25`W	1200	Firn-area

Analysis of selected compounds:

Pesticides and metabolites:

The analysis contain the following older pesticides: Aldrine, bromophos, bromophos-ethyl, carbofenothione, chlordane, chlorfenvinphos, op´-DDD, pp'-DDD, op'-DDE, pp'-DDE, op'-DDT, op'-DDT, diazinone, dieldrine, dimethoate, endosulfane I, endosulfane II, endrine, fenitrothione, fonofos, alfa-HCH, beta-HCH, gamma-HC (lindan), delta-HCH, heptachlor, heptachlorepoxide, hexachlorbenzene, malathion, mirex, parathion, parathion-methyl, pentachlorphenol and tetrachlorvinfos.
Further we conducted analysis of the following pesticides and degradation products typically analysed when monitoring ground water used as drinking water in Denmark: Alachlor, atrazine, bentazone, bromoxynile, carbofurane, 4-chlor-2-methylphenole, 4-CPP, cyanazine, 2,4-D, 2,6-DCPP, DE-atrazine, DE-terbutylazine, DIP-atrazine, dicamba, dichlobenile, 2,6-dichlorbenzamide (BAM), 2,4-dichlorphenole, dichlorprop (2,4-DP), dinoseb, DNOC, ethofumesat, fenpropimorph, fluazifop-(p)-butyl, hexazinone, ioxynil, isoproturone, lenacile , MCPA, mechlorprop, metabenzthiazurone, metazachlore, metribuzine, pendimethaline, pirimicarb, propazine, propiconazole, propyzamide, simazine og terbutylazine. Detection limits for most pesticides are 0,002 µg/l.

PAH:

15 PAH´s, present on the EPA list, were selected: Naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)antracene, chrysene/triphenylene, benzofluoranthens (b+j+k), benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenz(ah)anthracene and benzo(ghi)perylene. With the exception of Naphthalene (0,02 µg/l) the detection limit is 0,002 µg/l.

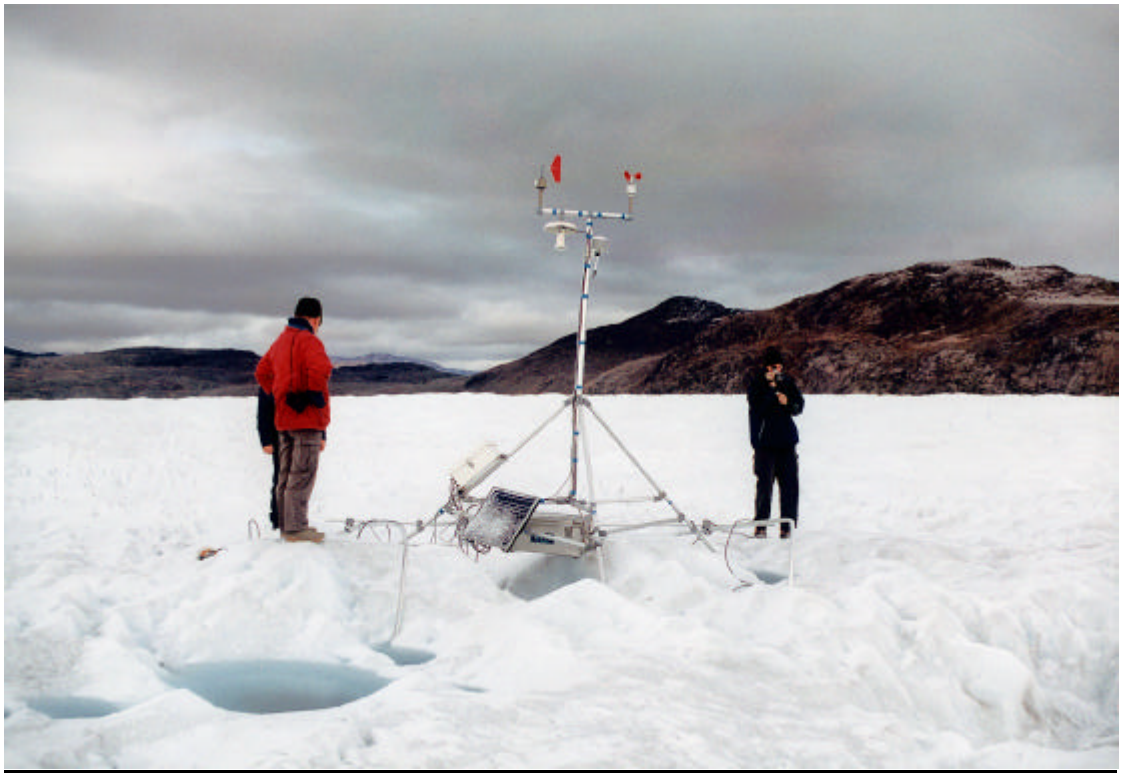
PCB:

Of the PCB´s the following 7 congeners are analysed for: PCB # 28, PCB # 52, PCB # 101, PCB # 118, PCB # 138, PCB # 153 og PCB # 180. Detection limits are 0,002 µg/l.

Methods of analysis:

Chemical analysis was conducted at Eurofins (Miljø-kemi) accredited to the analysis to most of the compounds. Samples were not filtered, as compounds present sorbed to particular matter should be included in the results. Pesticides were extracted in both acid and basic solutions containing dichlor-methane, followed by concentration steps and analysis by gaschromatography with mass-spectrometric detector by selective ion monitoring (GC/MS-SIM). Confidence of analysis RSD is 15%. At numbers less than 10 times the detection limit of the method, however, the RSD is up to 50%.

PAH´s and PCB´s were extracted in acid solution containing dichlor-methane, followed by concentration steps and analysis by gaschromatography with mass-spectrometric detector by selective ion monitoring (GC/MS-SIM). Confidence of analysis RSD is 15% for PCB and 12% for PAH. At numbers less than 10 times the detection limit of the method, however, the RSD is up to 50%.



Microbiological analysis:

Sampling procedures:

A frozen block of ice originating from glacier 1AH0200 was cut up in a freezer. In order to ensure aseptic handling of samples the work was done in a laminar flow bench and all tools used was sterilised prior to use. Sterile control samples was placed in the flow bench to detect possible contamination occurring during the handling of samples.

Viable micro-organisms:

Samples where melted in sterile glass containers and a subset was concentrated using sterile 0.2µm filters. The concentrated sample was hereafter spread on 4 different media (1/10 TSA, water agar, coliform petrifilm and VA petrifilm) and incubated at 4 different temperatures (30°C, 20°C, 5°C and -1°C). We used these different media in an attempt to cover the viable micro-organisms specific growth demands to the best possible extent. Likewise the different incubation temperatures are reasoned by a potential difference in temperature-optima and temperature-tolerance within the present population of viable micro-organisms. The colony development on the agar-plates was monitored over a period of 2 month. Further the concentrated samples were analysed by microscopy.

Melting and storage:

Subsets where transferred to sterile glass containers and stored both aerobically and anaerobically at 3 different temperatures (30°C, 20°C and 5°C). Anaerobic conditions were continually monitored in relevant subsets. Numbers of viable micro-organisms were monitored over a period of 18 days.

Characterisation of most frequent viable micro-organisms:

All isolates were frozen at -80°C and are stored at this temperature at GEUS. Selected isolates (selected by their ability to re-grow on 1/10 TSA at the incubation temperatures 5°C or 20 °C) were characterised by conventional methods (colony-morphology, -colour, and speed

of colony-development at the different incubation temperatures, assimilation- and enzyme-profile on the API 20 NE identification system (BioMerieux, France)). Further selected isolates will be characterised on the basis of 16S-ribosomal DNA sequences.

Results of chemical analysis:

Selected compounds:

We did not detect any traces of pesticides, PCB or PAH compounds in sample 3 originating from glacier 1AH0200. Detailed data from chemical analysis can be found in appendix 1.

In contrast, sample 4, originating from the firn-area at Narsarsuaq and consisting of snow from 2000 and 2001, contained a range of chemical contaminants. Sample 1 collected near the "road" leading to the VW test facility contained, not surprisingly, low concentrations of PAH´s probably emitted from car exhaust. Additionally the sample contained pentachlorphenol in concentrations near the detection limit as well as traces of dimethoat.

In sample 4 the content of pentachlorphenol was very high and therefore certain. Additionally all 15 PAH´s are detected and most of these in concentrations far higher than the detection limit. Concentrations of BAM and simazin, in contrast, are so low that they cannot be regarded as significant. The concentrations of the 3 PCB´s are also near the detection limit and therefore uncertain.

Presence of contaminants in Glacier 1AH0200 (sample 3).

Sample nr. /height above sea-level	Location/estimated age (years)	Pesticides Concentration in µg/l	PCB Concentration in µg/l	PAH Concentration in µg/l
3/ 200	Narsarsuaq/ >1000	*	*	*

* No presence of compounds detected.

Deposits:

The distribution of chlorinated organic compounds between air, snow, seawater and the marine mammal food chain is described in AMAP (1998).
The origin of the PAH´s can be both natural and anthropogenic. According to Masclet et al. (2000) there is the following connection between the specific PAH´s and their sources:

Combustion	PAH tracer
Coal	Fluoranthene
Fuel oil	Phenanthrene
Automobiles exhaust	Benzo (ghi)perylene, indenopyrene, coronene
Biomass burning	Pyrene, chrysene, coronene
Boreal forest fires	Retene

Masclet et al. (2000) states flouranthene and pyrene as the most common PAH´s in cities. They also find these as the most common PAH´s deposited in snow. The mean annual figure is given as 1800 pg/g (~1.8 µg/kg).

Results of microbial analysis:

Viable bacteria:

The microbial analysis contained spreading and incubation of samples on a total of 80 agar-plates and 60 petrifilm. Spreading was done on 4 different media:

1) Water agar

Water agar is composed of agar and sterilised water. This media has an extremely low nutrient content and is normally used to isolate nutrient sensitive bacteria from oligothrophic environments.

2) 1/10 TSA

1/10 TSA is a general commercial media and according to our experience the best suited to isolate bacteria from environmental samples.

3) Aerobic count plate Petrifilm (3M)

Aerobic count plate Petrifilm (AC) is another general media specifically designed to do commercial analysis of colony forming units in the food industry.

4) Coliform Petrifilm (3M)

Coliform petrifilm is a media designed to count coliform (and potentially pathogenic) bacteria.

In an attempt to cover the temperature demands of the potentially present bacteria all samples were incubated at 4 different temperatures 30°C, 10°C, 5°C and -1°C. CFU from samples using the different media and incubation temperatures are shown in the table below.

Isolated micro-organisms from glacier 1AH0200:

Media / temperature	CFU November	CFU December	CFU Januar	Total 13. Jan.
Water agar 30°C	0/ml	0/ml	-	0/ml
- - 10°C	0/ml	0/ml	0/ml	0/ml
- - 5°C	0/ml	1/ml	3/ml	4/ml
- - -1°C	-	0/ml	2/ml	2/ml
1/10 TSA 30°C	2/ml	2/ml	-	4/ml
- - 10°C	0/ml	46/ml	14/ml	60/ml
- - 5°C	1/ml	65/ml	9/ml	75/ml
- - -1°C	-	0/ml	13/ml	13/ml
C Petrifilm 30°C	0/ml	0/ml	0/ml	0/ml
- - 10°C	0/ml	0/ml	0/ml	0/ml
- - 5°C	0/ml	0/ml	0/ml	0/ml
- - -1°C	0/ml	0/ml	0/ml	0/ml
AC Petrifilm 30°C	10/ml	0/ml	-	10/ml
- - 10°C	13/ml	18/ml	-	31/ml
- - 5°C	2/ml	14/ml	-	16/ml
- - -1°C	1/ml	5/ml	12/ml	18/ml

Within the first 14 days a total of only 6 colonies had appeared. This corresponds to below 0,5 CFU/ml. As a comparison the maximum number of allowed CFU in Danish tap water is 200 /ml.

We did not, however, stop the incubation at this point and registered the appearance of new colonies in the following weeks in December and January. After 2 month the incubation was terminated and after this period of time the total number of CFU was still lower than the previously stated maximum limit applied to Danish tap water.
Incubating samples at 30°C resulted in the lowest number of CFU, while incubating samples at 10°C or 5°C resulted in highest numbers. This corresponds well to the anticipated generally low temperature tolerance of micro-organisms from ice samples. Further we did not, at any time or at any incubation temperature, detect colonies on the coliform petrifilm. This indicates a minimal risk of presence of potentially pathogenic bacteria.

Data from aerobic count plate petrifilm 30°C, 10°C, 5°C as well as water agar 30°C and 1/10 TSA 30°C from January are missing due to the overgrowth of fungi. However, the numbers of CFU´s was at no time prior to this overgrowth of fungi in proximity of the limiting value of 200 CFU/ml applied to Danish tap water.

Microscopic analysis of samples:

Using microscopy it was not possible to detect a single bacterial cell corresponding to the presence of less than 1000 cells/ml. This method is not very sensitive, however, together with the plate counts it rejects the presence of a large number of "viable but non-culturable"-bacteria in the samples.

Bacterial growth after deposition:

We conducted melting and deposition under both anaerobic (without oxygen) and aerobic (with oxygen) conditions at 30°C, 10°C and 5°C. Spread of samples on agar media under anaerobic conditions did not show growth of bacteria in samples deposited under anaerobic conditions. Samples melted and deposited under aerobic conditions, showed great differences between different temperatures. Deposition at 30°C did not result in growth of micro-organisms, while samples deposited at 10°C contained approximately 30.000 CFU/ml after 18 days. Samples deposited at 5°C showed substantial growth of bacteria and numbers

reached 200.000 CFU/ml. These investigations are conducted using 3 repetitions and control samples have shown that no contamination has occurred.
It is therefore probable that bacteria present in the ice can use the few nutrients present in the melted ice and reach relatively high concentrations after longer deposition events at low temperatures.

Characterisation of isolates:

Isolated colonies were frozen in 50% Glycerol and stored at -80°C. By doing this bacteria can be kept in a living but inactive state for long periods of time. The frozen strains from the samples are currently stored at this temperature at GEUS.

All isolates were re-plated on 1/10 TSA at 5°C and 20°C. It was not possible to re-grow all the isolates and only 10 isolates from the 1AH0200 ice sample re-emerged under these conditions. This could be due to differences in original isolation conditions and re-growth conditions, or possibly several isolates have not survived the freezing procedure.

The re-grown bacteria were subject to characterisation by several different methods; Colony-morphology, -colour, speed of colony development at different temperatures, microscopy (to identify yeast), assimilation- and enzyme-profile on API 20 NE). Additionally selected isolates will be characterised on the basis of their 16 S rDNA sequence.

Characterisation data from the 10 strains isolated from glacier 1AH0200 are shown in tables below. Several strains did not grow at the high temperature (20°C) or grew faster at the low temperature (5°C). Further there was a clear correlation between original isolation temperature and ability to grow at specific re-growth temperature. Several strains were pigmented and formed bright yellow colonies.

Conventional characterisation of re-grown isolates.

				Incubation-temperature			
	Original isolation conditions			**20 °C**		**5 °C**	
GEUS nr.	Origin	Temp.	From media	Growth	Colour	Growth	colour
1	ICE	5	TSA	No		Very small	
22	ICE	10	TSA	No		Medium	white
29	ICE	30	AC-pertrifilm	Small	White	No	
30	ICE	30	AC-pertrifilm	Small	Yellow	No	
31	ICE	30	TSA	Large	Yellow	No	
71	ICE	5	AC-pertrifilm	No		Small	white
88	ICE	30	AC-pertrifilm	Small	White	No	
174	ICE	10	VA	No		Very small	
194	ICE	-1	VA	No		Small	white
202	ICE	-1	AC-pertrifilm	No		Very small	

Characterisation on API 20 NE:

As a laboratory routine working with new microbial strains we assessed the possible presence of potentially human pathogenic strains among the isolated microorganisms. The strains were tested using the API 20 NE test system. This system is designed to identify a range of bacteria.

Characterisation on API 20 NE

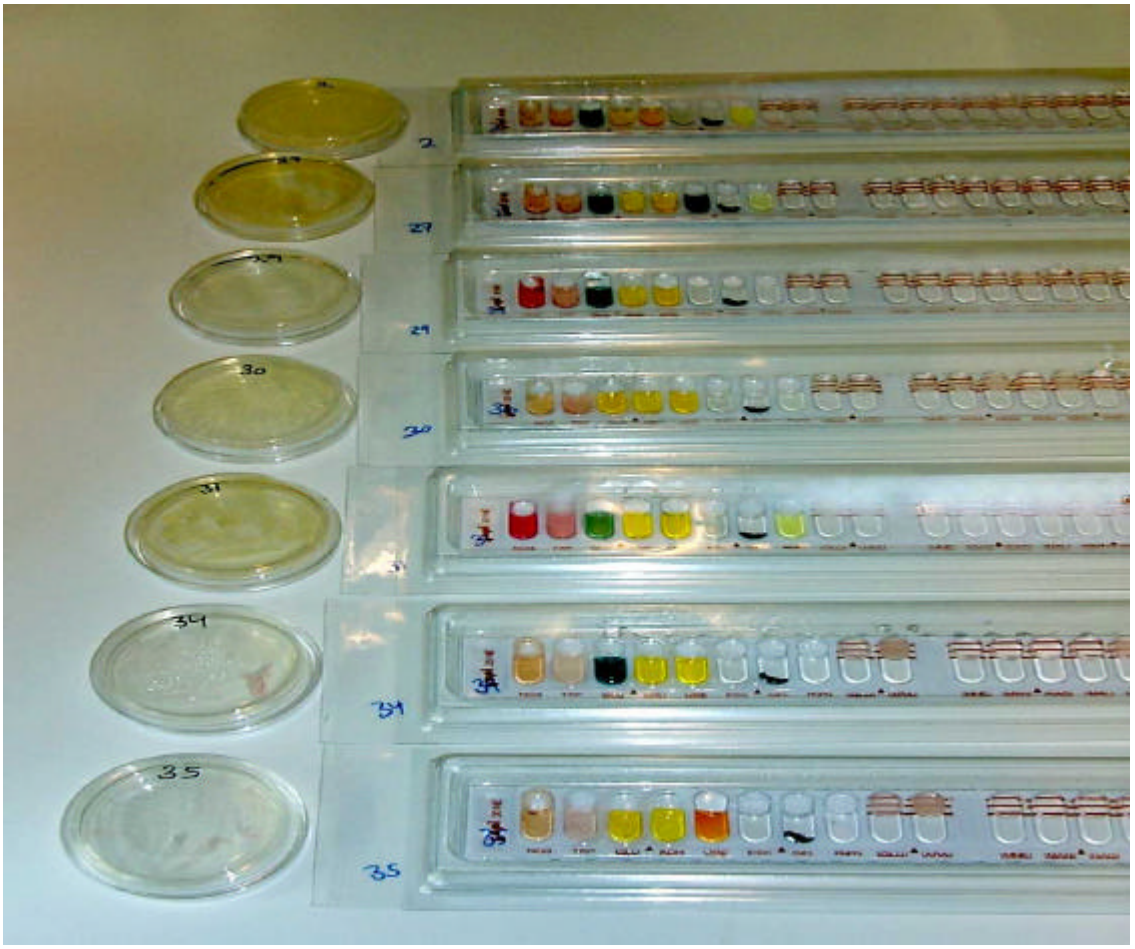
	Original isolation conditions		**Identity on API-20 NE/microscopy**		**Identity by 16S sequencing**
GEUS nr.	Origin	Temperature	Isolation media		
1	ICE	5	TSA	Unknown (no growth)	
22	ICE	10	TSA	*Yeast* (Microscopy)	To be identified by 18S
29	ICE	30	AC-pertrifilm	Unknown (no growth)	*Sphingomonas* sp. BF14
30	ICE	30	AC-pertrifilm	Unknown (no growth)	
31	ICE	30	TSA	Unknown (limited growth)	
71	ICE	5	AC-pertrifilm	Unknown (no growth)	*Herbaspirillum Seropedicae*
88	ICE	30	AC-pertrifilm	Unknown (no growth)	
174	ICE	10	VA	Unknown (no growth)	
194	ICE	-1	VA	Unknown (no growth)	
202	ICE	-1	AC-pertrifilm	Unknown (no growth)	

Unfortunately the isolates from the ice could not be identified as only very little growth occurred. However this signify the presence of human pathogens as improbable.

Sequencing:

The only possibility for identification of the isolates then is using molecular techniques sequencing the rDNA sequences from the bacteria. These sequences gives a genetic "fingerprint" of the bacteria and the use of this technique is thoroughly tested and approved. These investigations are presently being conducted on NOVOzymes and are expected to be due in the end of February.

Until present 2 strains have been sequenced and identified as close relatives of *Sphingomonas* sp. BF14 (strain 29) and *Herbaspirillum seropedicae* (strain 71), respectively. These strains are not known to be pathogenic to humans.



Conclusion:

Chemical investigations:

We did not detect any contaminants in significant amounts present in samples originating from glacier 1AH0200, representing ice of an estimated age of more than a thousand years.
In samples taken at other locations, representing ice from present date (collected in connection with a separate project, see appendix), significant concentrations of PAH´s have been detected.

Microbiological investigations:

The microbiological investigations found a low number of viable micro-organisms present in the ice. These organisms show a remarkable adaptation to life in cold environments and some show interesting features such as growth at low (as well as extremely low, -1°C) temperatures and absence of growth at higher temperatures. In connection to the possible use of the ice for human consumption it is generally reassuring that the present micro-organisms do not grow at high temperatures. Further we did not detect any coliform bacteria and the isolates did not show any relation to known human pathogenic strains assessed by API 20 NE or sequencing.

If the ice is melted and deposited under aerobic conditions a growth of micro-organisms is anticipated even, or especially, if stored at low temperature. Ice melted and deposited under anaerobic conditions do not support bacterial growth.

References:

AMAP (1998) Assessment Report: Artic Pollution Issues. Artic Monitoring and Assessment Program. ISBN 82-7655-061-4

Christner C.B., E. Mosley-Thomsen, L.G. Thomsen, V. Zagorodnov, K. Sandman, J.N. Reeve, (1999). Recovery and identification of viable bacteria immured in glacial ice. ICARUS **144**, 479-485.

Enge E.K., G. Christensen, R. Kallenborn, D. Herzke & T. Skotvold (2001) Persistent organic pollutants in snow and melt water from Bjørnøya (Bear Island). Organohalogen compounds, P217, 52: 467-470.

Gordon, D.A., J. Priscu, S. Giovannoni, (2000). Origin and phylogeny of microbes living in permanant antartic lake ice. Microbial Ecology **39**, 197-202.

Jacobsen, C.S. (2000). GEUS rappo*rt 2000/86*.

Kallenborn R., G. Christensen, E.K. Enge, D. Herzke, M. Schlabach & T. Skotvold (2001) Persistent organic pollutants in ambient air and fog at Bjørnøya (Bear Island). Organohalogen compounds 52: 413-416

Masclet, P., Hoyau, V., Jaffrezo, J.L. Cachier, H. (2000). Atmospheric Environment, **34**, 3195-3207.

Skidmore, M. L., J.M. Foght, M.J. Sharp, (2000). Microbial life beneath a high arctic glacier Appl. Environ. Microb. **66**, 3214-3220.

U.S. Geological Survey (1995) Fact Sheet FS-152-95)

Villa, S., Maggi, V., Bolzacchini, E., Rindone, B., Vighi, M., Belloli, R. (2000). Prepr. Ext. Abstr. ACS Natl. Meet., Am. Chem. Soc., Div. Environ. Chem., **40** (1), 397-399.

Appendix:

Analyserapport

Depositions projekt

Analyse af is og sne

Rekvirent: **GEUS**
Gitte Felding
Danmarks og Grønlands Geologiske Undersøgelse
Thoravej 8
2400 København NV

Dato: 7. maj 2002

Udført af: MILJØ-KEMI, Dansk Miljø Center A/S
Holsbjergvej 42, DK-2620 Albertslund

Nis Hansen
udviklingschef

Yvonne Simonsen
sektionsleder

Appendiks-fortsat

Prøvemateriale

Prøverne var mærket:

- Prøve 1
- Prøve 2
- Prøve 3
- Prøve 4

Prøverne er modtaget i rilsanposer og er opbevaret i fryser (-20°C) indtil analyserne er påbegyndt.

Prøverne er efter aftale analyseret efter følgende program:

- Pesticider
- PAH
- PCB

For enkeltstoffer se resultattabellerne side 4-9.

Analyserne er udført i perioden 08.03.2002 - 03.05.2002.

Appendiks-fortsat

Analysemetoder:

For at minimere kontaminering har prøverne efter udtagelsen af fryser henstået et par timer, og det yderste lag er tøet, hældt fra og ikke medtaget i analysen.
Derefter er prøven delt og en delprøve taget i analyse. Analyserne er udført på den homogeniserede delprøve (totalprøve).

Pesticider (sur) i vand (MK-2270)

Princip: Vandprøven pH justeres og ekstraheres 3 gange med dichlormethan. Det samlede ekstrakt inddampes. Ekstraktet methyleres og analyseres ved gaskromatografi med massespektrometrisk detektor (GC/MS-SIM). Pentachlorphenol medtages efter denne metode.

Analyseusikkerhed: RSD 15%, ved værdier mindre end 10 gange metodens detektionsgrænse dog op til 50%.

Pesticider (basisk) i vand (MK-2271)

Princip: Vandprøven pH justeres og ekstraheres 3 gange med dichlormethan. Det samlede ekstrakt inddampes og analyseres ved gaskromatografi med massespektrometrisk detektor (GC/MS-SIM).

Analyseusikkerhed: RSD 15%, ved værdier mindre end 10 gange metodens detektionsgrænse dog op til 50%.

PAH og PCB i vand (MK-2260)

Princip: Prøven gøres sur til pH 2 og ekstraheres med dichlormethan. Efter inddampning analyseres ekstraktet ved gaskromatografi med massespektrometrisk detektor ved selektiv ion monitering (GC/MS-SIM).

Analyseusikkerhed: RSD 12% for PAH og 15% for PCB, ved værdier mindre end 10 gange metodens detektionsgrænse dog op til 50%.

Appendiks-fortsat

Is- og sneprøver

Enhed: µg/liter	Prøve 1	Prøve 2	Det.grænse
Pesticider:			
Aldrin	-	-	0,002
Bromophos	-	-	0,002
bromophos-ethyl	-	-	0,002
Carbofenothion	-	-	0,002
Chlordan	-	-	0,002
Chlorfenvinphos	-	-	0,002
op'-DDD	-	-	0,002
pp'-DDD	-	-	0,002
op'-DDE	-	-	0,002
pp'-DDE	-	-	0,002
op'-DDT	-	-	0,002
op'-DDT	-	-	0,002
Diazinon	-	-	0,005
Dieldrin	-	-	0,002
Dimethoat	0,014	-	0,01
endosulfan I	-	-	0,005
endosulfan II	-	-	0,005
Endrin	-	-	0,005
Fenitrothion	-	-	0,002
Fonofos	-	-	0,002
alfa-HCH	-	-	0,002
beta-HCH	-	-	0,002
gamma-HC (lindan)	-	-	0,002
delta-HCH	-	-	0,002
Heptachlor	-	-	0,002
Heptachlorepoxid	-	-	0,002
Hexachlorbenzen	-	-	0,002
Malathion	-	-	0,002
Mirex	-	-	0,002
Parathion	-	-	0,005
parathion-methyl	-	-	0,002
Pentachlorphenol	0,002	-	0,002
Tetrachlorvinfos	-	-	0,002

-: Mindre end den anførte detektionsgrænse.

Appendiks - fortsat

Is- og sneprøver

Enhed: µg/liter	Prøve 1	Prøve 2	Det.grænse
Pesticider (fortsat):			
Alachlor	-	-	0,002
Atrazin	-	-	0,002
Bentazon	-	-	0,002
Bromoxynil	-	-	0,002
Carbofuran	-	-	0,02
4-chlor-2-methylphenol	-	-	0,003
4-CPP	-	-	0,002
Cyanazin	-	-	0,002
2,4-D	-	-	0,002
2,6-DCPP	-	-	0,002
DE-atrazin	-	-	0,002
DE-terbutylazin	-	-	0,002
DIP-atrazin	-	-	0,01
Dicamba	-	-	0,02
Dichlobenil	-	-	0,02
2,6-dichlorbenzamid (BAM)	-	-	0,002
2,4-dichlorphenol	-	-	0,02
dichlorprop (2,4-DP)	-	-	0,002
Dinoseb	-	-	0,002
DNOC	-	-	0,01
Ethofumesat	-	-	0,002
Fenpropimorph	-	-	0,002
Fluazifop-(p)-butyl	-	-	0,002
hexazinon	-	-	0,005
Ioxynil	-	-	0,002
Isoproturon	-	-	0,002
Lenacil	-	-	0,005
MCPA	-	-	0,002
mechlorprop	-	-	0,002
metabenzthiazuron	-	-	0,002
metazachlor	-	-	0,002
metribuzin	-	-	0,005

-: Mindre end den anførte detektionsgrænse.

Appendiks - fortsat

Is- og sneprøver

Enhed: µg/liter	Prøve 1	Prøve 2	Det.grænse
Pesticider (fortsat):			
pendimethalin	-	-	0,005
pirimicarb	-	-	0,002
propazin	-	-	0,002
propiconazol	-	-	0,005
propyzamid	-	-	0,002
Simazin	-	-	0,002
Terbutylazin	-	-	0,005
PAH:			
naphthalen	0,025	-	0,02
acenaphthylen	-	-	0,002
acenaphthen	-	-	0,002
Fluoren	0,002	-	0,002
phenanthren	-	-	0,005
anthracen	-	-	0,002
Fluoranthen	0,002	-	0,002
pyren	-	-	0,002
benz(a)antracen	-	-	0,002
chrysen/triphenylen	0,003	-	0,002
benzofluoranthener (b+j+k)	0,003	-	0,002
benzo(a)pyren	-	-	0,002
Indeno(1,2,3-cd)pyren	-	-	0,002
dibenz(ah)anthracen	-	-	0,002
benzo(ghi)perylen	-	-	0,002
PCB:			
PCB # 28	-	-	0,002
PCB # 52	-	-	0,002
PCB # 101	-	-	0,002
PCB # 118	-	-	0,002
PCB # 138	-	-	0,002
PCB # 153	-	-	0,002
PCB # 180	-	-	0,002

-: Mindre end den anførte detektionsgrænse.

Appendiks-fortsat

Is- og sneprøver

Enhed: µg/liter	Prøve 3	Prøve 4	Det.grænse
Pesticider:			
aldrin	-	-	0,002
bromophos	-	-	0,002
bromophos-ethyl	-	-	0,002
carbofenothion	-	-	0,002
chlordan	-	-	0,002
chlorfenvinphos	-	-	0,002
op'-DDD	-	-	0,002
pp'-DDD	-	-	0,002
op'-DDE	-	-	0,002
pp'-DDE	-	-	0,002
op'-DDT	-	-	0,002
op'-DDT	-	-	0,002
diazinon	-	-	0,005
dieldrin	-	-	0,002
dimethoat	-	-	0,01
endosulfan I	-	-	0,005
endosulfan II	-	-	0,005
endrin	-	-	0,005
Fenitrothion	-	-	0,002
Fonofos	-	-	0,002
alfa-HCH	-	-	0,002
beta-HCH	-	-	0,002
gamma-HC (lindan)	-	-	0,002
delta-HCH	-	-	0,002
heptachlor	-	-	0,002
heptachlorepoxid	-	-	0,002
hexachlorbenzen	-	-	0,002
malathion	-	-	0,002
mirex	-	-	0,002
parathion	-	-	0,005
parathion-methyl	-	-	0,002
pentachlorphenol	-	6,1	0,002
Tetrachlorvinfos	-	-	0,002

-: Mindre end den anførte detektionsgrænse.

Appendiks - fortsat

Is- og sneprøver

Enhed: µg/liter	Prøve 3	Prøve 4	Det.grænse
Pesticider (fortsat):			
alachlor	-	-	0,002
atrazin	-	-	0,002
bentazon	-	-	0,002
bromoxynil	-	-	0,002
carbofuran	-	-	0,02
4-chlor-2-methylphenol	-	-	0,003
4-CPP	-	-	0,002
cyanazin	-	-	0,002
2,4-D	-	-	0,002
2,6-DCPP	-	-	0,002
DE-atrazin	-	-	0,002
DE-terbutylazin	-	-	0,002
DIP-atrazin	-	-	0,01
dicamba	-	-	0,02
dichlobenil	-	-	0,02
2,6-dichlorbenzamid (BAM)	-	0,009	0,002
2,4-dichlorphenol	-	-	0,02
dichlorprop (2,4-DP)	-	-	0,002
dinoseb	-	-	0,002
DNOC	-	-	0,01
ethofumesat	-	-	0,002
Fenpropimorph	-	-	0,002
Fluazifop-(p)-butyl	-	-	0,002
hexazinon	-	-	0,005
Ioxynil	-	-	0,002
Isoproturon	-	-	0,002
Lenacil	-	-	0,005
MCPA	-	-	0,002
mechlorprop	-	-	0,002
metabenzthiazuron	-	-	0,002
metazachlor	-	-	0,002
metribuzin	-	< 0,05 *	0,005

-: Mindre end den anførte detektionsgrænse.

*: Forhøjet detektionsgrænse på grund af interferens.

Appendiks-fortsat

Is- og sneprøver

Enhed: µg/liter	Prøve 3	Prøve 4	Det.grænse
Pesticider (fortsat):			
pendimethalin	-	-	0,005
pirimicarb	-	-	0,002
propazin	-	-	0,002
propiconazol	-	-	0,005
propyzamid	-	-	0,002
Simazin	-	0,003	0,002
Terbutylazin	-	-	0,005
PAH:			
naphthalen	-	0,025	0,02
acenaphthylen	-	0,014	0,002
acenaphthen	-	0,028	0,002
Fluoren	-	0,033	0,002
phenanthren	-	0,41	0,005
anthracen	-	0,033	0,002
Fluoranthen	-	0,65	0,002
pyren	-	0,35	0,002
benz(a)antracen	-	0,11	0,002
chrysen/triphenylen	-	0,30	0,002
benzofluoranthener (b+j+k)	-	0,51	0,002
benzo(a)pyren	-	0,17	0,002
Indeno(1,2,3-cd)pyren	-	0,16	0,002
dibenz(ah)anthracen	-	0,041	0,002
benzo(ghi)perylen	-	0,17	0,002
PCB:			
PCB # 28	-	-	0,002
PCB # 52	-	0,002	0,002
PCB # 101	-	0,003	0,002
PCB # 118	-	-	0,002
PCB # 138	-	0,002	0,002
PCB # 153	-	-	0,002
PCB # 180	-	-	0,002

-: Mindre end den anførte detektionsgrænse.

Appendiks – fortsat

Isolerede mikroorganismer; Vækst af isolater og indledende karakterisering:

	Oprindelige isoleringsforhold			Vækket ved 20 grader på 1/10 TSA			Vækket ved 5 grader på 1/10 TSA		
Fryserørs nr.	Hvorfra	Temp. i C	Fra medie	Vækst 7dg.	Farve	Kommentar	Vækst 7 dg.	Farve	kommentar
70	F	30	TSA	store		transparente	små		transparente
81	F	30	TSA	store		transparente	små		transparente
82	F	30	TSA	store	gule				
83	F	30	TSA	store	gule				
84	F	30	TSA	store	gule				
86	F	30	TSA	store	gule				
87	F	10	C-pertrifilm	store	hvide		små	hvide	
163	F	-1	TSA	store	lyserøde		store	lyserøde	
167	F	-1	AC-pertrifilm	store	hvide		store	hvide	
168	F	-1	AC-pertrifilm				små	transparente	
169	F	-1	AC-pertrifilm	små	hvide	transparente	små	hvide	transparente
179	F	10	VA-TSA		højgul				
1	IS	5	TSA				meget små		
22	IS	10	TSA				mellem	hvide	
29	IS	30	AC-pertrifilm	små	hvide				
30	IS	30	AC-pertrifilm	små	gule				
31	IS	30	TSA	store	gule				
71	IS	5	AC-pertrifilm				små	hvide	
88	IS	30	AC-pertrifilm	små	hvide				
174	IS	10	VA-TSA						
194	IS	-1	VA - TSA				små		hvide
202	IS	-1	AC-pertrifilm						
2	S.S.	5	TSA	små	hvide		små	hvide	
27	S.S.	10	TSA	store	højgule		store	højgule	
34	S.S.	30	AC-pertrifilm	bittesmå	røde	hårde			
35	S.S.	30	AC-pertrifilm	bittesmå	røde	hårde			
56	S.S.	10	AC-pertrifilm	store	solgul		små	solgul	
57	S.S.	10	AC-pertrifilm	store	lyserøde		store	lyserøde	
58	S.S.	10	AC-pertrifilm	store	hvide		små	hvide	
59	S.S.	10	AC-pertrifilm	store	orange		små	gu-lorange	
60	S.S.	10	AC-pertrifilm		hvide	transparent	små	hvide	transparent
61	S.S.	10	AC-pertrifilm	store	orange		små	orange	
67	S.S.	5	TSA	store	orange		små	orange	
68	S.S.	5	TSA	store	orange		små	orange	
69	S.S.	5	AC-pertrifilm	store	lyserøde		store	lyserøde	
80	S.S.	5	AC-pertrifilm		hvide	transparent		hvide	transparent
110	S.S.	10	AC-pertrifilm	små	hvide				
113	S.S.	10	TSA	stor	gul	1			
141	S.S.	5	AC-pertrifilm				små	hvide	
162	S.S.	-1	AC-pertrifilm	store	lyserøde		store	lyserøde	
191	S.S.	-1	AC-pertrifilm	små	hvide		små	hvide	
192	S.S.	-1	AC-pertrifilm				gule	små	

Appendiks – fortsat

Isolerede mikroorganismer; vækst af isolater på API 20 NE strimler:

Fry-serørs nr.	Hvorfra	Temp. i C	NO3	TRP	GLU	ADH	URE	ESC	GEL	PNG	GLU A	R A	MNE	MAN	NAG	MAL	GNT	CAP	ADI	MLT	CIT	PAC
70	F	30	-	-	-	-	-	+	-	+	+	+	+	+	+	+	+	-	-	+	-	+
81	F	30	-	-	-	-	-	+	-	+	+	+	+	+	+	+	+	-	-	+	-	-
82	F	30	-	-	-	-	-	-	+	-	+	+	+	+	+	+	+	-	+	+	+	+
83	F	30	-	-	-	-	-	-	+	-	+	-	+	-	-	+	-	-	+	+	+	+
84	F	30	-	-	-	-	-	-	+	-	+	-	+	-	-	+	-	-	+	+	-	+
86	F	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
87	F	10	-	-	-	-	-	+	-	+	+	+	+	+	+	+	+	-	-	-	-	+
163	F	-1	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
167	F	-1	-	-	-	-	+	+	-	+	-	-	-	-	-	+	-	-	-	-	-	-
168	F	-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
169	F	-1	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
179	F	10	-	-	-	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+
1	IS	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22	IS	10	-	-	-	-	-	+	-	-	+	+	+	+	+	+	+	-	+	+	+	-
29	IS	30	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
30	IS	30	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
31	IS	30	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-
71	IS	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
88	IS	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
174	IS	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
194	IS	-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
202	IS	-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	S.S.	5	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
27	S.S.	10	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
34	S.S.	30	-	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	+	+	-	-
35	S.S.	30	-	-	+	-	+	-	-	-	+	+	-	-	-	-	+	-	+	+	-	-
56	S.S.	10	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
57	S.S.	10	-	-	-	-	-	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-
58	S.S.	10	+	-	-	-	+	+	-	+	+	-	+	-	-	-	-	-	-	+	-	-
59	S.S.	10	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
60	S.S.	10	+	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-
61	S.S.	10	-	-	-	-	-	+	-	+	+	+	+	-	+	+	+	-	-	+	-	-
67	S.S.	5	-	-	-	-	+	-	-	-	-	-	-	+	+	-	+	-	+	+	+	-
68	S.S.	5	-	-	-	-	+	-	-	-	+	+	-	+	+	-	-	-	-	+	+	-
69	S.S.	5	-	-	+	-	-	+	-	-	+	+	+	+	+	+	+	-	-	-	-	+
80	S.S.	5	+	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
110	S.S.	10	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
113	S.S.	10	-	-	-	-	-	-	+	-	+	+	+	-	-	+	+	-	+	+	-	+
141	S.S.	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
162	S.S.	-1	-	-	-	-	-	+	-	+	-	+	+	+	+	+	+	-	-	-	-	-
191	S.S.	-1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
192	S.S.	-1	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-

Appendiks-fortsat

Isolerede mikroorganismer; Ompodning og identifikation ved sekventering:

Fryserørs nr.	Hvorfra	Temp. i C	Vækst 2 dg. 20°C	Vækst 2 dg. 5°C	Vækst 10 dg. 5°C	Identifikation
70	F	30	+	(+)		Rape rhizosphere Bacterium
81	F	30	+	(+)		Rape rhizosphere Bacterium
82	F	30	+			Micrococcus luteus
83	F	30	+			
84	F	30	+			
86	F	30	+			
87	F	10	+	(+)		Gær (Mikroskopi)
163	F	-1	+	(+)		Gær (Mikroskopi)
167	F	-1	+	+		Gær (Mikroskopi)
168	F	-1		+		
169	F	-1	(+)	(+)		
179	F	10	+			
1	IS	5			+	
22	IS	10		+		Gær (Mikroskopi)
29	IS	30	((+))			Sphingomonas sp. BF14
30	IS	30	(+)			
31	IS	30	(+)			
71	IS	5			lille vækst	Herbaspirillum seropedicae
88	IS	30	((+))			
174	IS	10			+	
194	IS	-1		+		
202	IS	-1			+	
2	S.S.	5	(+)	(+)		Gær (Mikroskopi)
27	S.S.	10	+	+		Sphingomonas sp. BF14
34	S.S.	30	(+)			
35	S.S.	30	(+)			
56	S.S.	10	(+)	(+)		Sphingomonas sp. BF14
57	S.S.	10	+	+		
58	S.S.	10	((+))			Arthrobacter sp.
59	S.S.	10	+	+		
60	S.S.	10	(+)	(+)		Arthrobacter sp.
61	S.S.	10	+	(+)		Sphingomonas sp. M3C203B-B
67	S.S.	5	(+)	(+)		
68	S.S.	5	(+)	(+)		
69	S.S.	5	+			Gær (Mikroskopi)
80	S.S.	5	+	+		
110	S.S.	10	(+)		+	
113	S.S.	10	+			
141	S.S.	5				
162	S.S.	-1	+	+		Gær (Mikroskopi)
191	S.S.	-1	(+)	(+)		
192	S.S.	-1	(((+)))	(+)		Bacterium CS117 Cryobacterium (ikke-filamentøs actinomycet)