LIFE IN ANCIENT ICE: A WORKSHOP

(sponsored by the National Science Foundation)

Organizers

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LIFE IN ANCIENT ICE WORKSHOP

FINAL REPORT

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EXECUTIVE SUMMARY

A workshop was held at the Westin Salishan Lodge, Gleneden Beach, Oregon from 30 June to 3 July, 2001. Thirty-five participants from Australia, Canada, Denmark, Germany, Israel, Russia, the USA, and the UK attended the forum that included a mixer, two days of seminars, a day of discussion, and conversations during meals and breaks. Twenty additional authors were involved. The interactions among the participants was stimulating and will hopefully lead to future meetings, collaborations, and research. The intensity and excitement were evident, indicating that the study of life in ancient ice and permafrost will rapidly progress in the future. The following is a synopsis of the workshop, including recommendations to address contamination, methods, and future research.

The general objective of this workshop was to bring together experts studying ice, permafrost, ancient life, biological preservation, evolution, and astrobiology to assess current and future research that would extend our knowledge of life in ancient frozen matrices. The anticipated outcomes of this workshop were to: **1.** Discuss current and future research on life in ancient ice and permafrost; **2.** Present recommendations to NSF and NASA on future research directions and needs; **3.** Generate interest among researchers and the public around the world on this subject by producing a book based on the workshop presentations and another book for the lay public (both to be published by Princeton University Press), making the abstracts and other information available to the public via the Internet.

The workshop also served as a forum for discussion of methodological approaches to microbial detection in ancient matrices. Several approaches have been used. For eukaryotic microbes the following methods have been used: culturing to obtain viable species, light and electron microscopy for identification by morphology of remains, and molecular amplification using the polymerase chain reaction (PCR) followed by DNA sequencing and comparison to extant species sequences. For the bacteria and archaea, culturing to obtain viable species, microscopy, measurement of metabolic indicators, and PCR amplification followed by sequencing of specific regions of their genomes and comparison to sequences of extant species have been used. For the viruses, transmission electron microscopy (TEM) to quantify major virus types, virus isolation from viable bacterial cultures, tissue cultures to identify viable viruses, and PCR amplification and sequencing for identification and characterization of specific human, animal, and plant viruses have been used. Thus, a clear picture of changes in fungal, protist, bacterial, archaeal, and viral species diversity in time and space are now possible. When working with ancient microbes and nucleic acids, the potential for contamination is very real. Issues of contamination and decontamination were discussed in detail. Through the workshop presentations and discussions, recommendations for improvement of methods and for future research were synthesized.

The general recommendations of the group are as follows:

1. Replication of the results in one or more additional laboratories is vital to the studies of organisms and nucleic acids in ancient ice and permafrost.

2. Indicator organisms and nucleic acids should be used as controls in all assays.

3. Quantitation should be performed and controls included in all stages of the work with ancient samples.

4. Further discussion, collaboration, and cooperation are needed prior to future projects on ice and permafrost on Earth and elsewhere.

5. Those of us who work on the microbiology of natural cryoecosystems need to become formally organized. One possible name for the group might be the "Subzero Biology Working Group."

GOALS

The specific goals for this workshop were to:

1. Bring together top researchers in diverse disciplines who work with ancient ice and permafrost to present their latest information for discussion and debate.

2. Present to NSF and NASA the needs and recommendations for research within a framework of the future envisioned by the participants at the workshop.

3. To generate public, scientific, and educational interest and excitement for further study of microorganisms in ancient ice, including ice and permafrost on Earth and elsewhere in the solar system, by producing two books (one based on the workshop) and an Internet web site.

VISION STATEMENT

Biological research of ancient ice and permafrost is integral to studies of microbial evolution, organism longevity, mechanisms of biological degradation, biochemical cycling, microbial gene transfer, genome recycling, medical microbiology, epidemiology, and astrobiology. This research should be expanded, refined, and widely utilized and publicized.

OUTCOMES

Outcomes are listed for each goal listed above.

1. A more coherent picture of the current knowledge and future needs has emerged by bringing together the top researchers in the studies of the origin of life, ice, permafrost, life in ancient ice, and astrobiology. Discussions in a conducive atmosphere stimulated the participants to formulate new ideas and potential collaborations. The workshop venue was such that the participants spent the majority of their time with each other to ensure maximum opportunity for interaction. The interactions among participants lead to an overwhelming agreement that more collaboration and continued discussion and meetings are desired. These should be initiated and planned within the coming year. It is our hope that the discussions and collaborations generated at the workshop will continue long after the end of the workshop. It appears that they will. Three focus groups met on the last day. The first discussed contamination and decontamination. This is an extremely important issue because contemporary organisms and nucleic acids can easily contaminate ancient specimens. It is crucial to address this topic and try to come to some consensus on the best ways to isolate and identify ancient organisms while avoiding contemporary contaminants. Contamination of other planets, moons, and other bodies by Earth-originating space probes is a real possibility. Conversely, decontamination and isolation of potential pathogens is an important issue if extraterrestrial ice specimens are brought back to Earth, since there will be health considerations as well. The second focus group dealt with methods to detect and identify microorganisms and their nucleic acids. Methods on ice coring, transport, examination, processing, culturing, and molecular biology were discussed. The third group discussed future research directions and/or initiatives.

2. Copies of this final reports are to be presented to NSF, NASA, and each participant in the workshop.

3. One book, edited by Scott O. Rogers and John D. Castello, will be based on the workshop presentations. It will include chapters written by the participants based on their workshop presentations, as well as the proceedings and recommendations from each of the focus groups (probable publication date 2002). A second book, authored by S.O. Rogers and J.D. Castello, will be written for a general audience, and will thus reach a different population of readers. Both books will be published by Princeton University Press (probable publication date 2002). Abstracts of the presentations and proceedings will be made available on an Internet web site. This will be linked to the NSF web site as well as others. By making the information available to NSF, NASA, on the Internet, and in the books, interest should be generated among scientists and the public worldwide on the study of life in ancient ice.

PROGRAM RATIONALE

The program for the workshop consisted of six sessions, discussions in three focus groups, and introductory and concluding remarks. The organization of the sessions was meant to begin with details about ice, then to proceed to specifics on nucleic acids and microorganisms entrapped in ice. The next three sessions provided details on groups of organisms, followed by a session on some of the evolutionary implications of the results, as well as an introduction into astrobiology. For each session, some of the top researchers made presentations and submitted manuscripts. The following is a brief description of each session:

Session 1: Sources and Characteristics of Ice. The physical characteristics of ice, its preservative qualities, sources of ice on Earth, and details of glacier-lake interactions were outlined.

Session 2: Longevity, Isolation, and Contamination. Descriptions and discussions were presented on protocols for isolating and characterizing organisms, nucleic acids, and other biomolecules from ice, as well as the theoretical limits of longevity of organisms and nucleic acids in ancient matrices, including ice and permafrost.

Session 3: Prokaryotes in Ancient Ice. The status of recent research of prokaryotes in ancient ice and permafrost was presented.

Session 4: Eukaryotes in Ancient Ice. The status of the recent research of eukaryotes in ancient ice and permafrost was presented.

Session 5: Viruses in Ancient Ice. The status of the recent research of viruses in ancient ice was presented, as well as a review of the animal and human viruses expected in recent and ancient ice.

Session 6: Evolution and Astrobiology. The effects of encasement in ice on rates of microbial evolution, the origin of life, and the search for life beyond Earth were discussed.

Focus Groups. Three focus groups were convened at the conclusion of the sessions. Participants were assigned to specific groups based on their expertise and interest. The groups discussed specific topics and the chair of each group then reported to the entire collection of participants. One group discussed matters of contamination and decontamination. Specific issues covered included the best ways to decontaminate the outside of a sample in order to isolate only organisms and nucleic acids from the interior of the ice sample. Another issue was the decontamination procedures that might be used on a space probe. A second group discussed the current methodologies used for extracting and transporting cores, as well as for isolating and identifying microorganisms, their nucleic acids, and other signs of biological activity. The third group discussed some general and specific research areas that require more effort and focus, and thus need to be strengthened in the future, as well as to examine new research directions.

INTRODUCTION AND BACKGROUND

We walk on ice, drive on ice, cool with ice, eat ice, and drink ice meltwater, but we seldom think about what is in the ice and meltwater. In your tap water there are can be tens of thousands of viable microbial cells per milliliter, representing hundreds of species. After freezing and thawing the water, there are approximately one-third as many cells and species. Similar or higher numbers of active viruses may be present. Thus,

freezing kills only a proportion of microbes in a given volume of water. Ice and permafrost on Earth contain huge numbers of living and dead microorganisms. It is estimated that close to 10^{19} viable microbes melt out of glaciers worldwide each year. In times of warming, the numbers can be much higher. Most of this ice (84.1%, or over 12.5 million km^2) is lying on top of the continent of Antarctica. Another 12.1% (1.8 million km^2) covers Greenland. About 2.2% (335,000 km^2) covers all of the other arctic islands. The remainder of the ice is scattered in the various mountain ranges around the world, for a total coverage of about 15 million km^2, or about 10% of the land area. Therefore, ice and permafrost are huge repositories of viable microbes, some of them having been frozen for hundreds of thousands to millions of years.

The extensive chronological sequence and availability of glacial ice (Lorius et al. 1979, 1985; Wolff and Peel 1985) provide an excellent framework for additional studies on microbial longevity and environmental influences on biotic diversity. With global sampling, glacial ice also can provide a temporal and spatial assessment of worldwide microbial biodiversity. The initial report of microorganisms in ancient ice was from a Russian investigator in 1775 (from Egorowa 1931). Over the next 1.5 centuries, only a few reported studies on microorganisms in ice were published, and primarily related to human health issues (e.g. Fränkel 1886). However, during the past 30 years has it been a topic of concentrated research, and during the past decade molecular biology methods have been used to study microorganisms in ancient ice.

Initially, these studies concentrated on bacteria. While bacteria remain the primary study organisms in ancient ice and permafrost, plants (algae and mosses, as well as spores and pollen from vascular plants), protists, fungi, archaea, and viruses have also been studied. Ice and permafrost are excellent matrices for the preservation of microorganisms and their nucleic acids. This has wide-ranging implications. First, the longevity of these microbes and their constituents preserved in frozen materials can be studied. Second, microbe concentrations and microbial diversity can be studied along various time courses. Third, these time courses can be correlated with geological, climatological, anthropogenic, and other characteristics. Fourth, methods can be developed to study the microorganisms (some of them unique to ice and permafrost) isolated from these matrices. Fifth, since ice and permafrost have been found on other bodies within the solar system, the methods developed can be adapted to search for life (or signs thereof) in the ice, permafrost, and in water oceans beyond Earth.

A surprising number and variety of microorganisms have been recovered from the interior of glacial ice cores dating back beyond 400,000 years, and permafrost samples dating back into the millions of years. Researchers have performed systematic surveys for bacteria, fungi, protists, vascular plants (as pollen), and viruses in samples of these ancient ice and permafrost samples. It is evident that ice and permafrost are natural air-sampling and preservation matrices, that have trapped wind-transported microorganisms through geological epochs. These organisms (representing taxa that are endemic to the polar regions, as well as exotics from temperate and tropical regions) originated from aerosols generated by sea wave action, wind-borne pollen and soil particles, infected plant surfaces, and many other sources. Thus, ice and permafrost provide global sources for microorganisms that present a view of both contemporary and ancient eukaryote, archaeal, bacterial, and viral diversity.

Profiles of successively older ice and permafrost strata will extend temporal and spatial assessments of microbial biodiversity to geologic time frames. The microorganisms might then be utilized in subsequent studies as bioindicators of global changes in climate, and geologic and human activity. Polar ice and permafrost have entrapped what was available in the past from the atmosphere. Temperate and tropical taxa are represented, in addition to the polar and desert taxa. In addition, species detected across a continuous chronological sequence can be utilized to study phylogeny, mechanisms of longevity and evolution, dormancy, environmental changes, and methods for microbial preservation. Finally, the protocols developed, tested, and recommended may contribute to future searches for microorganisms in extraterrestrial ice (e.g., ice from the Moon, Mars, Europa, comets, etc.). Therefore, for comparative purposes it will be essential to know what microorganisms are present in ancient Earth ice and permafrost. Additionally, since the Earth is

bombarded daily with dust, rocks, and water from space, there is a possibility that microorganisms are being deposited onto the surface of the Earth with this extraterrestrial matter.

• Characterization of Bacterial and Fungal Ice Communities

There is an inherent interest in knowing how long microorganisms can remain viable (Gest and Mandelstam 1987). Viable thermoactinomycete endospores have been found in deep mud cores in excess of 1,000 years old (Cross and Attwell 1974). Viable microbes have been isolated from 320 year-old herbarium specimens (Sneath 1962), thermophilic bacteria have been isolated from ocean basin sediment cores estimated to be 5,800 years old (Bartholomew and Paik 1966), and bacteria have been recovered from Roman archaeological sites 1,900 years old (Seaward et al. 1976). Empirically based mutation rates that result in detrimental effects on spore viability have been used to estimate the half-life of *Bacillus subtilis* at 7,000 years (for matrices other than amber). Assuming an exponential death rate, a large population of viable spores would be detectable after several hundred-thousand and possibly for several million years (Gest and Mandelstam 1987; Cano and Borucki 1995). These expectations assume that the immediate environment protects the spores from ultraviolet irradiation, oxidation, and chemical damage common with hydrated nucleic acids. Investigators have isolated viable bacteria from ice cores at Vostok Station in Antarctica, some of which were deposited more than 400,000 years ago (Abyzov et al. 1979, 1982a, 1982b, 1982c, 1983, 1986; Karl et al. 1999; Kudriashov et al. 1977, 1978; Priscu et al. 1999), and from Siberia, up to 8,000 years old (Shi et al. 1997). Viable bacteria and fungi, as well as viral nucleic acids, from ice cores of less than 500 to over 400,000 ybp have also been reported (Castello et al. 1999; Catranis and Starmer 1991; Ma et al. 1997, 1998, 1999, 2000; Rogers et al. 1999, and unpublished data). Theoretical expectations on the longevity of bacterial spores had previously placed the limit for recovering viable bacterial spores at about 200,000 years (Gest and Mandelstam 1987; Sneath 1962). However, reports of the isolation, growth, and DNA characterization of 25 to 40 million year-old bacteria from amber and a 250 million-year-old bacterium from a salt crystal have pushed the theoretical limit substantially higher for some species entrapped in specific matrices (Cano and Borucki 1995; Vreeland et al. 2000).

Both exotic microorganisms and those endemic to polar regions have been recovered from soil, air, snow, seawater, and sea ice of the polar regions (Cameron, et al. 1972, 1974; Dmitriev et al. 1997; Johnson et al. 1978; Ling and Seppelt 1990; Staley et al. 1989b). Garrison et al. (1986) reviewed the results of numerous studies of microbial assemblages and maximum algal biomass in polar sea ice. Abyzov (1993) summarized studies on recovery of viable fungi and bacteria from the antarctic ice sheet. Many reports on recovery of viable microorganisms from deep subsurface environments have been restricted to those from deep mud cores (Cross and Atwell 1974), deep aquifers, and other subsurface sediments (Balkwill 1989, Fliermans and Balkwill 1989, Balkwill et al. 1989, Beeman and Suflita 1990). More recently, bacteria, fungi, protists, viruses and other organisms have been characterized from ancient ice and permafrost (Abyzov 1993; Catranis and Starmer 1991; Dmitriev et al. 1997; Ma et al. 1997, 1998, 1999, 2000; Rogers et al. 1999). The earliest mycological research of circumpolar fungi (Rostrup 1888; Cardot et al. 1910; Lind 1910a, 1910b, 1924a, 1924b, 1926; Larsen 1931), and fossil fungi (Seward 1898; Meschinelli 1902; Pia 1927; Wolf and Wolf 1947; Tiffney and Barghoorn 1974; Pirozynski 1976; Stubblefield and Taylor 1988) has been primarily taxonomic. Morphological, substrate associations, and geographical distribution studies of arctic fungi have been conducted (Kobayasi et al. 1967; Kobayasi 1982). Sun et al. (1978) investigated the qualitative and quantitative distribution of fungi in soil and air at antarctic dry valley sites. They did not obtain "obligate psychrophiles", but their work was a significant advance in the collection and interpretation of morphological data. Recent reports of fungi in ice from both poles has confirmed that the entrapped fungi are arriving from around the world (Catranis and Starmer 1991; Ma et al. 1997, 1998, 1999, 2000; Rogers et al. 1999, and unpublished data).

Antarctic yeasts have been studied extensively. Reports on their occurrence range from fortuitous observation to in-depth studies of their ecology and life histories. DiMenna's (1966a, 1966b) reports were among the early descriptions of unique species of yeasts in Antarctica. Fell (1974, 1976, Fell and Phaff 1967, Fell et al. 1969, 1973, Fell and Hunter 1974, Fell and Tallman 1982, Kurtzman and Fell 1997) also described several new species with life cycles that helped develop new approaches to the study of basidiomycetous yeasts. Vishniac described several new species from antarctic soils and cryptoendolithic communities (Vishniac and Hampfling 1979, Vishniac 1985, Baharaeen and Vishniac 1982, Vishniac and Baharaeen 1982). While a variety of ascomycetous and basidiomycetous species have been isolated from Antarctica (Atlas et al. 1978, Sugiyama 1969, Goto et al. 1969, diMenna 1966a, 1966b, Sinclair and Stokes 1965), the majority of new and unique species are basidiomycetes, suggesting their particular value as phylogenetic tools in the studies we propose.

• Characterization of Viral Ice Communities

Because viable bacteria and fungi have been isolated from ice cores, the viruses that infect them are likely to be present as well. Many viruses (or phage) of bacteria (Ackerman and DuBow 1987) and fungi (Lemke 1979) have been reported. However, no bacteria or fungi isolated from glacial ice have been examined for virus infection. At low multiplicity of infection, most phage become lysogenic within the host genome and are undetectable by conventional means (Prescott et al. 1996). However, active replication of the virus, which will allow its detection, can be induced by stressing the cells. In the laboratory, this is accomplished by exposure of lysogenized cells to ultraviolet irradiation or other agents that damage host DNA (Prescott et al. 1996). Recent studies have revealed unexpectedly high numbers of viruses (10^9 virions/ml) in the oceans, where they infect phytoplankton and bacteria, effectively controlling primary productivity (Bergh et al 1989, Proctor and Fuhrman 1990, Milligan and Cosper 1994, Noble and Fuhrman 1996). Indeed, up to 70% of all marine bacteria are estimated to be infected with lysogenic viruses, and the top one milliliter of the oceans may contain 10^{30} virus particles (Prescott et al. 1996). These results strongly suggest that viruses also reside in ancient viable bacteria isolated from glacial ice.

Although viruses or virus-like particles are reported in many genera of fungi, well-described and characterized viruses have been reported from few genera (Ghabrial 1994). Of these, only *Aspergillus* and *Penicillium* have been isolated from glacial ice (Catranis and Starmer 1991; Ma et al. 1997, 1998, 1999, 2000; Rogers et al. 1999). Unfortunately, unlike viruses of bacteria, plants, and animals, none of the fungal viruses likely to infect fungi isolated from ice has been well characterized at the molecular level.

Certain highly stable plant, animal, and human viruses are likely to be present in glacial ice. Some groups of plant viruses (e.g., Tobamoviruses) include members with a worldwide distribution and wide host range that occur in plant debris (Zaitlin and Israel 1975), soil (Büttner and Nienhaus 1989), fresh water (Jacobi and Castello 1991, Koenig 1986), and marine ecosystems (Kegler et al. 1994). The stability of the Tobamoviruses, for example, is legendary among virologists; tobacco mosaic Tobamovirus (TMV) has been launched into outer space and was still infectious upon return (Orlob and Lorenz 1968). Infectious tomato mosaic Tobamovirus (ToMV) has been reported in fog and clouds (Castello et al. 1995). It is also present in glacial ice cores from Greenland (Castello et al. 1999; Rogers et al. 1999). Sequence analysis of a 244 bp fragment from these samples indicates that a diverse assemblage of ToMV strains exists in the ice (Castello et al. 1999; Rogers et al. 1999).

Many enteric viruses are present in soil, water, and air in which they can spread and initiate disease outbreaks (e.g., parvovirus, foot-and-mouth disease virus, poliovirus, influenza virus, coxsackie and echoviruses, hepatitis A virus, etc.) (Rao and Melnick 1986). Therefore, it seems likely that some of the more stable of these viruses also may have found their way into glacial ice. However, despite the likelihood of many different virus types in glacial ice, a systematic survey to detect them has not been conducted. Viruses

surround us in the soil, air, and water. But how might they enter glacial ice? The dynamics of aerial transport and survival of human and plant pathogens in water droplets have been studied extensively from the earliest days of microbiology (for reviews see: Fitt et al. 1989, Atlas and Bartha 1987, Riley and O'Grady 1961). Studies of infectious ToMV in clouds (Castello et al. 1995) suggest that glacial ice viruses originate from frequent and varied aerosolizations. Sources of airborne viruses include: water fall sprays, rain drops, sea spray and surf (Baylor et al. 1977), hurricanes, tornadoes, volcanic activity, dust storms, soil (Büttner and Nienhaus 1989), waste discharge and sanitary treatment (Shuval et al. 1989), infected animals and their feces (Grant et al. 1994, Gloster et al. 1982), windblown plant debris (Banttari and Venette 1980), agricultural activities, etc. Although individual virions are small enough (10-450 nm) to remain airborne nearly indefinitely, they are not likely to occur free of other materials. For example, when ejecta droplets evaporate, the viruses will remain associated and stabilized on nonspecific colloidal organic and mineral debris. Therefore, they ultimately will settle out or become incorporated into snowflakes or rain drops. However, the effects of turbulent dispersal on phage, plant, animal, and human viruses are little known. The dynamics of microbe deposition by snow, rain, fog, and clouds are equally obscure.

Atmospheric circulation over Antarctica allows air exchange with lower latitudes (Abyzov 1993). Microbes transported on dust and precipitation become embedded in ice that forms from falling snow. Direct sampling of the interior of Antarctica has repeatedly demonstrated microbes characteristic of more northerly latitudes (Cameron et al. 1972). Therefore, glaciers have entrapped at least some aerosolized microorganisms and viruses from ancient atmospheres, and thus are a natural air sampling mechanism and repository from which to assess ancient microbial diversity on a global scale.

Many questions arise regarding the occurrence of viruses in glacial ice. Why should we expect to find them there? How did they get there? What concentrations and geographical/temporal distributions are observed? Why? Are they infectious? Why haven't they been observed in previous ecological studies? Can their distribution over time be used as a bioindicator of global change? Of changes in human development? Can they be used to study rates of viral evolution? Is ice a reservoir for ancient pathogenic viruses, bacteria, and fungi that can be released in times of global warming? These and other questions will be the focus of future research once the identity and distribution of the viruses and other organisms present in ancient ice are determined.

• Community Profiles and Climatic Changes

Changes in relative abundances of specific ancient organisms from ice and permafrost can be used to formulate correlations between microorganism distributions and environmental characteristics (climatic conditions, vulcanism, etc.) at each of the time points. These comparisons might not only yield simple correlations to microbial blooms and epidemics, but the characteristics of these community changes might be used in conjunction with climatology, as well as in the detection of pollutants, ozone depletion, acid rain, and other anthropogenic and naturally-caused phenomena. If identical fungi/algae/bacteria/viruses occur in ice of similar age from both polar regions, then they are likely to have been globally distributed. It is a long-held supposition that microbial species are freely dispersed among comparable habitats. However, it was reported that although several different bacteria obtained from the north and south polar regions were remarkably similar, sequencing of 16S rRNA indicated they were distinct species (Staley 1989; Service 1997), suggesting that extant polar bacterial species are endemic to one pole or the other. But has this always been so? What about airborne nonendemic fungi, bacteria, and viruses? If not, a potentially significant direction for future research lies in using ancient microorganisms as bioindicators of global change.

The climate can change dramatically within relatively short periods of time. For example, the last glacial maximum (between ca. 25,000 and 18,000 years ago), and the last deglaciation (ca. 14,000 to 8,000 years ago) caused shifts in plant, animal, and human populations (Betancourt et al. 1990, 1991; Spaulding 1985,

1990, 1991; Spaulding et al. 1983; Spaulding and Graumlich 1986). During the last deglaciation in North America, winds from the equatorial Pacific Ocean moved far inland creating tropical climates in California, Nevada, Utah, Wyoming, and beyond. Such changes may be correlated with changes in populations of pathogenic viruses and fungi entrapped in ice, but this has not been investigated. Climatic fluctuations appear to be quite common, as evidenced by frequent dramatic increases in formation of icebergs and snow melts in Greenland. These have been correlated with fluctuations in rainfall and temperature in the continental USA over the past tens of thousands of years (Benson et al. 1996; Phillips et al. 1996).

• Future Research

As early as 1973, it had been suggested that Antarctica could be used as a model for Martian terrain. (Vishniac and Mainzer 1973). Given current technology, it is possible to search for microorganisms in ancient Martian matrices, comets, ice on the Jovian moon Europa (and others), and in lunar ice and other frozen substrates. Work with Earth ice will contribute to development of the proper protocols and methods. It is essential to know what microorganisms are present in ancient Earth ice for comparative purposes. McKay (1993) discussed the importance of studying polar ecosystems to test theories and develop methods to search for extraterrestrial life. We may also discover that extraterrestrial microbes, or microbes returning to Earth after being previously ejected by a bolide impact, have been deposited in the ice on Earth. The future of research in ancient ice and permafrost is bright and multifaceted. It will likely lead to many important and valuable outcomes in the near and distant future. These will be in the areas of astrobiology, evolutionary biology, glaciology, geology, microbiology, molecular biology, pharmacology, and others. This was the backdrop for the "Life in Ancient Ice" workshop.

PROGRAM

• June 30, 2001 (Saturday)

Attendees arrived at Salishan Lodge and Resort at Gleneden Beach, Oregon.

8:00 pm Welcome and Mixer at Salishan

• July 1, 2001 (Sunday)

6:00-7:30 BREAKFAST

INTRODUCTORY REMARKS

7:45 Welcome / Introductions / Development of the Workshop
Scott O. Rogers, Department of Biological Sciences, Bowling Green State University, Bowling Green Ohio, USA

7:55 Objectives and Format of Workshop
John Castello, EFB, SUNY ESF, Syracuse, New York, USA

SESSION 1: SOURCES AND CHARACTERISTICS OF ICE

8:00 **What are the sources of ice for biological studies?**
Joan Fitzpatrick, U.S. National Ice Core Laboratory, U.S. Geological Survey, Denver, Colorado, USA

8:30 **Linkages between ancient ice and subglacial lakes**
Robin E. Bell, Lamont-Doherty Earth Observatory, Columbia University, Palisades, New York, USA

SESSION 2: LONGEVITY, ISOLATION, AND CONTAMINATION

9:00 **DNA Damage, Longevity and Authenticity in Fossil Matrices**
Hendrik Poinar Max-Planck-Institut für evolutionäre Anthropologie, Leipzig, Germany

9:30 **COFFEE BREAK**

10:00 **Reviving Organisms and Characterizing Nucleic Acids From Ancient Matrices**
Scott O. Rogers, Department of Biological Sciences, Bowling Green State University, Bowling Green, Ohio, USA

10:30 **The Culturable and Unculturable Prokaryotes in Ancient Permafrost**
James M. Tiedje[1] and Monica Ponder[2], [1]University Distinguished Professor and Director Center for [1,2]Microbial Ecology, Michigan State University, East Lansing, Michigan, USA

11:00 **Life in Solid Ice?**
P. Buford Price, Physics Department, University of California, Berkeley, California, USA

11:30 **Metabolic Activity of Microorganisms in Permafrost**
Elizaveta Rivkina[1] and Kyastas Laurinavichus[2], [1]Institute for Physiochemical & Biological Problems of Soil Science, and [2]Institute of Biochemistry & Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Moscow Region, Russia

12:00 **Quantification of Metabolic Activity Below the Freezing Point**
Imre Friedmann and Roseli Ocampo-Friedman, NASA-Ames Research Center, Moffett Field, California, USA

12:30 **LUNCH**

SESSION 3: PROKARYOTES IN ANCIENT ICE

2:00 **Common Features of Microorganisms in Ancient Specimens**
S.S. Abyzov, Institute of Microbiology, Russian Academy of Sciences, Moscow, Russia

2:30 **Recovery of Bacteria and DNA from Glacial Ices**
John Reeve, Department of Microbiology, Ohio State University, Columbus, Ohio, USA.

3:00 **Prokaryotic communities from Antarctic sea ice**
David S. Nichols, School of Agricultural Science, University of Tasmania, Hobart, Tasmania

3:30 **Ancient Bacterial DNA from Permafrost Soils**
Eske Willerslev[1], Anders J. Hansen[1], Ian Barens[2], Tina B. Brand[1], David Gilichinsky[3], Alan Cooper[2], and Peter Arctander[1], [1]Department of Evolutionary Biology, University of Copenhagen,

Copenhagen, Denmark, [2]Institute of Biological Anthropology, Oxford University, Oxford, UK, [3] Soil Cryology Laboratory, Institute for Physicochemical & Biological Problems of Soil Science, Russian Academy of Sciences

4:00 **COFFEE BREAK**

4:30 **Search for Bacteria in Antarctic Ice Using Epifluorescence**
Ryan Bay, Physics Department, University of California, Berkeley, California, USA

5:00 **Viable Phototrophs: Cyanobacteria and Green Algae from Permafrost Darkness**
Tatiana Vishnivetskaya[1], Ludmila Erokhina[2], Elena Spirina[1], and Anastasia Shatilovich[1], [1]Soil Cryology Laboratory, Institute for Physicochemical & Biological Problems of Soil Science, [2]Institute for Basic Biological Problems, Russian Academy of Sciences, Pushchino, Moscow Region, Russia

5:30 **Cyanobacterial Dynamics in Antarctic Lake Ice**
John Priscu, Department of Land Resources and Environmental Sciences, Montana State University, Bozeman, Montana USA

6:30 **DINNER**

• July 2, 2001 (Monday)

6:00-7:30 BREAKFAST

SESSION 4: EUKARYOTES IN ANCIENT ICE

7:30 **Frozen In Time - The Diatom Record In Ice Cores From Remote Drilling Sites on the Antarctic Ice Sheets**
Davida E. Kellogg and Thomas B. Kellogg, Institute for Quaternary and Climate Studies, University of Maine, Orono, Maine, USA

8:00 **Non-Marine Diatoms in Antarctic Ice Cores: Provenance and Potential for Use as Indicators of Past Atmospheric Transport Paths**
Thomas B. Kellogg and Davida E. Kellogg, Institute for Quaternary and Climate Studies, University of Maine, Orono, Maine, USA

8:30 **Polar Yeasts**
Jack Fell[1] and Tom Starmer[2], [1]RSMAS, University of Miami, Miami, Florida, USA, [2]Biology Department, Syracuse University, Syracuse, New York, USA

9:00 **Ancient Eukaryotic (Yeast & Micromycetes) Communities Isolated from Permafrost**
Rushaniya Faizutdinova[1], Svetlana Ozerskaya[2], Galina Kochkina[2], Nataliya Ivanushkina[2], Natalia Suzina[2], Vitalii Duda[2], Vera Soina[3], and Lada Petrovskaya[4], [1]Soil Cryology Laboratory, Institute for Physicochemical & Biological Problems of Soil Science; [2]Institute of Biochemistry & Physiology of Microorganisms, Russian Academy of Sciences, Pushchino, Moscow Region; [3]Department of Soil Biology, Faculty of Soil Science, Moscow State University; and [4]Institute of Bioorganic Chemistry, Russian Academy of Sciences, Moscow, Russia

9:30 **COFFEE BREAK**

10:00 **Filamentous Fungi in Ice**
Li-Jun Ma[1], Cathy M. Catranis[2], W. Thomas Starmer[3], and Scott. O. Rogers[4]. [1]Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts. [2]EFB, SUNY ESF, Syracuse, NY, [3]Biology, Syracuse, University, Syracuse, NY, and [4]Biological Sciences, Bowling Green State University, Bowling Green, Ohio, USA.

10:30 **Biogenic particles and possible viable fungal spores from the transition ice above Lake Vostok, Antarctica.**
Ray Sambrotto and L. Burckle, Lamont-Doherty Earth Observatory, Columbia University, Palisades, New York, USA

SESSION 5: VIRUSES IN ANCIENT ICE

11:00 **Plant Viruses and Bacteriophage in Ice**
John Castello, Environmental and Forest Biology, State University of New York College of Environmental Science and Forestry, Syracuse, New York, USA

11:30 **Viral Pathogens of Animals in Ice**
Alvin Smith, College of Veterinary Medicine, Oregon State University, Corvallis, Oregon, USA

12:00 **Viral Pathogens of Humans in Ice**
Dany Shoham, Begin-Sadat Center for Strategic Studies, Bar-Ilan University, Ramat-Gan, Israel

12:30 **LUNCH**

SESSION 6: EVOLUTION AND ASTROBIOLOGY

2:00 **Recycling of Organisms and Genomes**
Scott O. Rogers, Biological Sciences, Bowling Green State University, Bowling Green, Ohio, USA.

2:30 **Origin and Evolution of Biotic Life**
Jose de la Torre, Monterey Bay Aquarium Research Institute (MBARI),Moss Landing California, USA

3:00 **Cryobiosphere: Ice and Permafrost as a Microbial Habitat on and Beyond Earth**
David Gilichinsky, Soil Cryology Laboratory, Institute for Physicochemical & Biological Problems of Soil Science, Russian Academy of Sciences

3:30 **COFFEE BREAK**

4:00 **Living Cells in Permafrost as an Object for Astrobiology Research**
Elena Vorob'eva, Department of Soil Biology, Faculty of Soil Science, Moscow State University, Russia

4:30 **Using Computer tomography for detection life in rocks, permafrost, and ice**
Alexandre I. Tsapin, Astrobiology Research Element, Jet Propulsion Laboratory, Pasadena, California, USA

5:00 **Water, Life and Fossils in Permafrost on Mars and Earth**
Dale Andersen[1] and Wayne Pollard[2], [1]NASA Ames, Moffett Field, California, USA, [2]McGill University, Montreal, QC, Canada

5:30 **Video Tape of Scuba Dive in an Antarctic Ice-Covered Lake**
Dale Andersen, NASA Ames, Moffett Field, California, USA

5:45 **Video Tape of Wind Patters Around Antarctica**
Thomas B. Kellogg, Institute for Quaternary and Climate Studies, University of Maine, Orono, Maine, USA

6:30 **DINNER**

• *July 3, 2001 (Tuesday)*

6:00-7:30 **BREAKFAST**

7:30 **Discussions in Focus Groups**
Focus Groups:
1. Contamination (Hendrik Poinar, chair)
2. Methodologies for Identifying Life Forms (Scott O. Rogers, chair)
3. Direction of Future Research (John Castello, chair)

9:30 **COFFEE BREAK**

10:00 **Discussions in Focus Groups / Write Recommendations**

12:00 **LUNCH**

2:00 **Reports from Focus Groups**
1. Contamination (Hendrik Poinar, chair)
2. Methodologies for Identifying Life Forms (Scott O. Rogers, chair)
3. Direction of Future Research (John Castello, chair)

3:30 **Closing Remarks**
Scott O. Rogers, EFB, Biological Sciences, Bowling Green State University, Bowling Green, Ohio, USA

3:40 **COFFEE BREAK**

6:30 **DINNER**

• *July 4, 2001 (Wednesday)*

6:00-7:30 **BREAKFAST**

Shuttle bus departed for the Portland International Airport

FOCUS GROUP REPORTS

• Contamination

Chair: Hendrik Poinar
Participants: Sabit Abyzov, Ray Sambrotto, Dany Shoham, Tatiana Vishnivetskaya, and Eske Willerslev (who presented the summary)

This group had several overall recommendations, as follows: 1. Replication of results in one or more additional laboratories is vital to confirm reports of organisms and nucleic acids in ancient matrices. 2. Indicator organisms and nucleic acids should be used throughout the process. For example, *Saccharomyces cerevisiae* cells and nucleic acids could be used as controls throughout processing of the ice, permafrost, culturing, and PCR (polymerase chain reaction) assays. 3. Assay sensitivities must be known. 4. Quantitation should be performed at all stages of the work. 5. Extreme attempts must be made to avoid contamination. While these measures are extensive and will undoubtedly slow research progress, repeatability and avoidance of all contamination is absolutely necessary for this work.

FIELD WORK

Several methods have been used to extract ice cores to minimize contamination. Although the avoidance of drilling fluids has been previously recommended by some researchers, dry drilling has some problems as well. The main problem is that there is more cracking of the ice when coring is done without fluids. With deeper cores, cracking increased due to the change in pressure as the core is brought to the surface. While drilling with fluids has been criticized, thorough comparisons of the two methods have not been performed. For example, systematic comparisons would include culturing and sequencing from each, including controls where indicators (organisms of differing size, nucleic acids of differing size, and small fluorescent molecules) are painted onto the outside surfaces of the cores. In this way, penetration of substances from the outside of the core (i.e., potential contaminants) into the interior could be quantified.

TRANSPORT TO LAB

While sterility of the surface of the cores is impossible in the field, additional contamination should be avoided. Sealing the cores in black plastic, wrapping in aluminum foil, and then placing them inside firm protective containers (PVC plastic or metal cylinders) is recommended. This will protect the cores from light, temperature changes, humidity variations, additional contamination (microbes, nucleic acids, etc.) and breakage during storage and transport to the lab. At this stage, one should assume that the outer surfaces of the cores are contaminated with bacteria, fungi, viruses, human cells, nucleic acids, etc.

IN THE LAB

A complete record for each core, including core-handling should be maintained. The cores should be clearly labeled and the top and bottom of the core sections should be marked. The core sections, permafrost sections, and samples originating from the sections should all be manipulated in a sterile hood or glovebox. The hoods and gloveboxes should be outfitted with a germicidal UV lamp that can be used to disinfect the surfaces prior to use. However, some organisms are highly resistant to UV-irradiation. Therefore, it is recommended that the surfaces also be disinfected with sodium hypochlorite (Clorox) and ethanol (optional) prior to working in the hood. Outer contaminants can be removed or eliminated by UV-irradiation and/or Clorox treatment. However, some organisms are not readily killed by UV-irradiation. Therefore, Clorox is recommended for many studies. Additionally, since UV-irradiation will penetrate the ice, shading of the central portion of the core section is necessary to avoid destruction of the organisms and nucleic acids inside the ice cores. This is not as much of a concern when studying permafrost. Heat has also been used to decontaminate the outer surface, but this may

not destroy all external contaminating organisms and molecules. Simple rinsing with water has also been employed. However, this is not recommended, since at best it simply dilutes external contaminants. In some studies, the core and permafrost sections are scraped with knives to remove the outer surfaces. Although in practice this appears to be effective there remains a possibility of contamination from the outer surfaces, since no sterilant is used.

After sterilizing the outer surface of the ice or permafrost sections with UV, Clorox, and/or scraping, samples are removed from the interior. This can be performed with knives, corers, drills/saws, or simply by melting, either at room temperature or in a refrigerator. When melting the core all of the equipment for melting must be brought to approximately the same temperature, so that the core will not crack or explode. The core sections can be melted directly in bottles, or shells of meltwater can be collected by placing the core section in a sterile funnel and allowing a specific volume of meltwater to be collected in successive bottles. In all cases, controls should be included where the outer surface of the ice core or permafrost section is subjected to culturing and DNA sequencing to determine what the outer contaminant are. Additionally, the ice cores and meltwater samples should be examined microscopically (light and electron, if possible) to determine any differences between the outer surfaces and the interior floral and faunal components.

CONTROLS

For Molecular Biology methods, typical positive and negative controls should be used. Controls to test the air filters should also be included. Culturing and PCR should be performed on all instruments, equipment, and solutions used in the entire process. Among the controls should be those that allow quantitation of the results.

• Methods for Identifying Life Forms

Chair: Scott Rogers
Participants: Dale Andersen, Ryan Bay, Anders Hansen, Thomas Kellogg, J. Smith, Elizaveta Rivkina, Jose de la Torre, and Sasha Tsapin (moderator for discussion on non-invasive methods)

In this group, there were many issues discussed, some in detail. Some specifics of the best methods were agreed upon, while others evoked debate. The consensus was that currently there exists a wide variety of methods available for the study of life and signs of life in ice and permafrost. However, there is room for improvement and much further discussion, collaboration, and cooperation are needed prior to developing future projects on ice and permafrost on Earth and elsewhere. This includes not only verbal and written communication among the individual researchers, but also a sharing of materials, including rare (and thus valuable) cores. Only through communication and cooperation can the best and most efficient use of these valuable cores be ensured.

Two matrices that were discussed in detail. The first is ice (in glaciers, sheets, sea, etc.) The second is permafrost, which includes soil below the freezing point of water, that may or may not contain significant amounts of ice. While ice generally contains organisms in low concentrations, permafrost usually has much high concentrations of microbes. Methods are many and variable due to the fact that there are methods for each purpose. There are hundreds of methods for culturing viable organisms depending on whether they are bacteria, archaea, eukarya, or viruses, and whether they are aerobes, anaerobes, psychrophiles, pathogens, etc. Procedures for decontamination of the outer portions of the ice and permafrost vary. Melting procedures, culture conditions, media, and other factors vary depending on the organisms sought. For non-culturable (currently) organisms, other methods, such as light and electron microscopy must be employed. However, the results are often equivocal. When seeking to characterize the nucleic acids and other biological molecules, the methods for handling the ice and permafrost are similar to those employed when culturing viable microbes. The methods for detecting biological molecules, especially nucleic acids, are very sensitive, such that great care is

needed to avoid contemporary contaminating molecules. Chemical analyses are also employed which utilize very different methods and precautions. Finally, when attempting to detect metabolic processes as indicators of living organisms, radioactive substrates are added to the samples. This requires special precautions to ensure that misleading results are avoided.

Two requests and recommendations were made with respect to the future NASA missions to Mars and elsewhere. First, the group should initiate and maintain contact with the NASA Planetary Protection Program. This program would be interested in the research and methods. In particular, the decontamination methods would be useful to this group. Additionally, other issues relating to the determination and reduction of contamination by manmade objects traveling to other planets and bodies, would be of interest to this group. The second recommendation was simply to share intellectual resources with others at NASA. There was a firm indication that the research and methods presented at the workshop could be valuable to those at in the Astrobiology program, as well as in other NASA programs.

Drilling methods were discussed in detail. There are three general drilling methods: thermal; mechanical with no fluid; and mechanical with fluids. While there was some concern that fluids used in drilling could penetrate, and thus contaminate, the core, the cores obtained by this method are usually in the best condition, having less distortion and fewer cracks. When drilling was accomplished using no drilling fluids, often the cores crack upon reaching the surface of the drill hole, primarily due to the sudden release of pressure. The deeper cores are under extreme pressure and when they are brought to the surface, the decrease in pressure causes them to crack from the inside outward. The thermal method also lead to cracking of the cores due to differences in temperature. Additionally, the cores were often misshapen, because the heated water remains in contact with the core sections for different amounts of time. The conclusion was that the mechanical method using drilling fluids provides cores in the best condition for use in most studies. The suggestions was made that a systematic comparison of the methods be performed, including controlled assays for microorganisms.

For each of the methods used to characterize specimens from ancient ice and permafrost, there are several general recommendations. Controls must be included in all studies. This would include numerous positive and negative controls. They should be employed in each step of the entire process. One suggestion was to paint indicators onto the outside of the ice core or permafrost section. Depending on the study, this could include indicator organisms, microparticles, nucleic acids, chemicals, or dyes. Another important control is to have the results replicated in at least one other independent laboratory.

Decontamination of the outer surfaces of the ice cores and permafrost sections has been accomplished with several methods. Chemical methods have been most often used with ice cores. The following have been reported: sodium hypochlorite (Clorox), chlorine gas, ethanol, ethylene oxide, hydrogen peroxide, methyl bromide, and ozone. The chemicals that are the most effective in killing all organisms and destroying nucleic acids and other biological molecules are oxidizers. The most effective oxidizers are sodium hypochlorite, chlorine, hydrogen peroxide, and ozone. An extensive amount of research on the decontamination efficacy, primarily related to human health issues. Sodium hypochlorite and chlorine are the most effective decontaminants. However, if part of the study is to measure chlorine in the sample, then these two cannot be utilized. Hydrogen peroxide and ozone are good alternatives, since hydrogen peroxide breaks down rapidly into water and oxygen gas, and ozone rapidly becomes oxygen gas. There are several other methods of decontamination: germicidal ultraviolet irradiation, cold plasma, shaving the outer surfaces, heating, and rinsing with water. However, ultraviolet irradiation fails to kill some organisms, even with extreme doses. Heating with flame may be effective for some matrices, but systematic studies are lacking. Cold plasma may be a good alternative to other methods, as it appears to be effective at burning most organic and many inorganic compounds. Shaving is the method of choice for permafrost samples. However, this method needs further testing to assure the avoidance of external contaminants. There was unanimous agreement that rinsing only with water was a poor choice for decontamination.

CULTURE METHODS

Most culture methods for bacteria, archaea, fungi, and other eukaryotes are specific for a group of organisms. Thus, these specifics were not discussed. However, the temperatures of incubation and series of temperatures were found to be crucial to successful culturing. Psychrophiles have been cultured at low temperatures, including temperatures below the freezing point of water. Temperatures of -8ºC to -12ºC have been used. To keep the medium from freezing, 20% glycerol is used. Other common temperatures used in culturing of organisms from ancient ice are 4ºC, 8ºC, 15ºC, 22ºC, or 37ºC, or a combination of these temperatures (for differing amounts of time).

MOLECULAR METHODS

Determination of the DNA sequence of a genomic region, following polymerase chain reaction amplification is the most common molecular method. It is also very sensitive, such that single contaminating molecules can lead to erroneous results. Thus, controls are crucial. DNA extraction prior to amplification can be avoided in some cases. There was some disagreement on this point, as some worried that the cells would not be broken and the DNA would not be available for PCR amplification. However, it was pointed out that in practice, amplicons are obtained and amplification is often more successful for dilute samples when DNA extraction is avoided. In general, the protocol that has the fewest manipulations while still yielding accurate results for a large proportion of samples is preferred. This should result in less chance for contamination. Whether DNA extraction is performed or not, intact cells must be broken. However, in the ice and permafrost, many cells may be broken in situ. Cell breakage can be accomplished via mechanical means, such as crushing with a pestle or between a glass slide and coverslip. The sample can be boiled in a microwave oven, water bath, or autoclave. Chemical breakage can also be used. Both enzymatic and hydrolytic methods can be used, but care must be take such that the DNA and/or RNA are not damaged. Finally, a pressure bomb was suggested as a way to break the cells. However, none had experience in using this method, so it is suggested that this be tested.

The genomic regions most often used are nuclear and organellar ribosomal DNA regions, as well as other mitochondrial and nuclear regions. The reason for using these regions is that there are large sequence databases available so that comparisons with a large number of extant taxa are possible. One new method being developed by a company utilizes rolling circle replication. This allows long pieces of DNA to be amplified and sequenced.

Because ice samples are so dilute, concentration is suggested. Filtration is one such method. However, some chemicals on the filters can be inhibitory to cell growth for culturing, and may inhibit some of the enzymes used in molecular biological protocols. Centrifugation is a method that avoids the addition of any inhibitory compounds. However, it can concentrate compounds already present in the sample that may be inhibitory in cultures or biochemical reactions. Also, this method is not useful in the case of organisms that are buoyant in water. Lyophilization is a third method that has been used in many cases. While it is very effective with small volumes of ice meltwater, it is not practical when using large volumes.

CHEMICAL METHODS

Many studies have been undertaken to search for life by examining samples using only chemical analyses. The first is simply to examine the elements and compounds released from the ice and permafrost. A second method is to look for zones of chemical disequilibria (redox reactions). A third method is to examine the samples for stable isotopes that are known to be incorporated by living organisms. With permafrost, the samples can be embedded in epoxy, sectioned, and then easily examined for isotopes. A fifth method is paleomagnetic studies of the ice and permafrost. A sixth method is to assay for organic compounds, such as nucleic acids, ATP, lipids, polysaccharides, and aromatic compounds (in general, if aromatics are absent, there can be no life). A seventh method is to examine the samples for the footprints of microbes, including tracks and fossils. Finally, mineral fabrics can indicate once present microorganisms, especially bacteria.

NON-INVASIVE METHODS

Using these methods, examination of the sample can be made without its physical destruction. The surface of a core or section can be examined with UV fluorescence, with a resolution of about 10-15μm. With Raman spectroscopy, resolution down to a few cells can be achieved. To peer deeper into the sections, ultrasonic methods can be used which employs phase interference to visualize inclusions in the ice. MRI (magnetic resonance imaging) is effective as long as paramagnetic substance levels are low. This method will not damage cells or their biological molecules. CT (computerized tomography) imaging can also be used. This method uses X-rays, and thus can be damaging to biological materials. The resolution at this point is not quite to the cellular level. However, using a synchrotron in conjunction with a CT scanner, one can perform elemental analysis on individual cells.

FINAL COMMENT

Two other comments were made that do not fit well with any other heading, but are nonetheless important. First, investigations should be initiated on DNA repair in the organisms that survive freezing conditions in ice and permafrost. They may be able to efficiently repair their DNA while entombed in these matrices. Second, there have been few reports of anaerobes from ice. The reason for this is probably due to the fact that ice always contains some dissolved oxygen. This is not true for permafrost, which is often anaerobic. Thus, anaerobes will probably be found in permafrost, but not in ice.

• Directions for Future Research

Chair: John D. Castello
Participants: Robin Bell, Jack Fell, Imre Friedmann, David Gilichinsky, Deneb Karentz, Davida Kellogg, David Nichols, Monica Ponder, Buford Price, John Priscu, Al Smith, and Elena Vorob'eva

The overall message is that we need to organize ourselves. A possible name might be: "The Subzero Biology Working Group " The objectives of the working group would be to organize and coordinate the research of our varied disciplines to a few deep cores from ice and permafrost (from the arctic and antarctic), and frozen rocks to thoroughly assess the microbial component (Fungal, Protista, Bacteria, Archaea, Viruses) using standardized methods. It should then be possible to draw meaningful conclusions about the entire microbial ecosystem from the same sources of material handled in the same manner. Such a task will require close coordination of research activities and frequent communication

Some questions/needs that we need to address: How long can life be preserved (permafrost is probably the best substrate for this purpose)? Is life below the freezing point active or dormant? If dormant, is it revivable? What life forms are present? Should this work be done before explorations for life on Mars? What is the relationship of life present to internal (temperature, presence of liquid water, dust, etc.) and external (historic, climatic, geologic) parameters? Can the quantity of life forms be related to internal and external parameters (mentioned above)? What is entering the ice and permafrost (air sampling)? Can this be related to what has been detected in old ice and permafrost? What is leaving the ice? Does genotype recycling occur? Is there a threat to humans, animals, plants, or microbes from pathogens? Is there lateral gene transfer (e.g. antibiotic resistance common feature of polar microbes) from ancient to contemporary bacteria? Are there any new gene products that can be extracted from ancient microbes (anticancer, antibiotic, industrially useful products)? Can we directly measure microbial mutation rates over long periods of time? Can the methods developed for detection of microbial life in frozen substrates in situ be directly applied to astrobiology research. What are the similarities and differences among different cold environments (ice, permafrost, subglacial lakes, cryptoendolithic communities, etc.)? What are the specific needs/questions of those working with: bacteria, protists, fungi, viruses, permafrost, and/or ice cores?

ORGANIZATION

We need to be represented on the ICWG (Ice Core Working Group), an ad hoc group that advises NSF-OPP (National Science Foundation - Office of Polar Programs) on issues relating to drilling and utilization of ice cores. The rationale for this is that biologists need to be involved in the planning/advisory stages of drill site locations, core handling, priority, and agenda setting. This also provides a forum for reporting our needs to ICWG, and its concerns to the biological community. Joan Fitzpatrick currently heads the group and they requested representatives from the biology community. John Priscu was nominated and elected. Scott Rogers will serve as a second representative.

PROPOSAL

The Subzero Biology Working Group (or whatever the final name) should meet again next year, with subsequent meetings to be determined at that time. John Castello was asked to head the group for the next year. He will approach NSF - OPP with a request to fund another workshop for the group next year. In addition, he will try to persuade NSF - OPP to fund a new initiative in subzero biology that includes both permafrost and polar ice possibly linked with paleoclimate work. We, as a group, need to link up with SCAR (Scientific Committee on Antarctic Research), an international group cochaired by John Priscu and Heinz Miller. They meet regularly and have their own website where meeting results and group activities are presented.

TIMELINE OR THE NEXT WORKSHOP (2002)

6/2001 - 12/2001	Initial invitations for speakers/attendees
	Proposal to NSF - OPP
	Secure funding commitment
	Contract workshop venue facility and others
	Initial travel arrangements
1/2002 - 4/2002	Finalize list of speakers/attendees/authors
	Finalize program
5/2002 - 6/2002	Finalize arrangements for facilities and travel
	Print programs, folders, nametags, etc.
7/2002	Workshop (3-4 days)
8/2002	Write and submit final report

Acknowledgements

We thank the National Science Foundation for funding, with special thanks to Polly Penhale for her support and valuable suggestions of speakers for the workshop. We also thank Deneb Karentz for her active participation in the workshop. Special thanks go to Tom Starmer who gave us the ideas and help to intiate this workshop. We thank all of the speakers and co-authors whose contributions made the workshop enjoyable. Their hard work will result in valuable research and literature on this topic.

Report submitted by Scott O. Rogers. Send comments and questions to: Scott O. Rogers, Department of Biological Sciences, 217 Life Sciences Building, Bowling Green State University, Bowling Green, Ohio, 43403; email: srogers@bgnet.bgsu.edu

REFERENCES CITED

Abyzov, S.S. 1993. Microorganisms in the Antarctic ice. Ch. 7, pp. 265-295, In: Friedman, E. I. (ed.). Antarctic Microbiology. John Wiley & Sons, Inc. New York. 634 p.

Abyzov, S.S., Babeva, I.P., Biryuzova, V.I., Kostrikina, N.A., and E.E. Azieva. 1983. Peculiarities of the ultrastructural organization of yeast cells from the interior of an Antarctic glacier. Biol. Bull. Acad. Sci. USSR. (Engl. Transl. IZV AKAD nauk SSSR Ser Biol) 10:539-545.

Abyzov, S.S., Bobin, N. E., and B.B. Kudriashov. 1986. Central antarctic glacier as an object of investigations of prolonged anabiosis of microorganisms in nature. Antarktiki; doklady komissii 25:202-208.

Abyzov, S.S., Bobin, N.E. and B.B. Kudriashov. 1979. Microbiological analysis of glacial series of central Antarctica. Akademiia nauk SSSR. Izvestia. Seriia biologicheskaia. 6:828-836.

Abyzov, S.S., Bobin, N.E. and B.B. Kudriashev. 1982b. Quantitative assessment of microorganisms in microbiological studies of Antarctic glaciers. Akademia nauk SSSR Investia Seriia biologicheskaia 6:897-905

Abyzov, S.S., Bobin, N.E. and B.B. Kudriashov. 1982c. Quantitative analysis of microorganisms during the microbiological investigation of antarctic glaciers. Biol. Bull. Acad. Sci. USSR. (Engl. Transl. IZV AKAD nauk SSSR Ser Biol) 9:558-564.

Abyzov, S.S., Lipenkov, V.Ya., Bobin, N.E., and B.B. Kudriashov. 1982a. Microflora of the central Antarctic glaciers and control methods of sterile isolation of the ice core for microbiological analyses. Akademia nauk microbiologicheskikh Seriia biologicheskaia .4:537-548.

Ackermann, H.W., and M.S. DuBow. 1987. Viruses of Procaryotes: Vol. 1. General Properties of Bacteriophages. CRC Press, Boca Raton, FL. 202 p.

Atlas, R.M., di Menna, M.E. and R.E. Cameron. 1978 Ecological investigations of yeasts in Antarctic soils. Terrestrial Biology III. Paper 2 in Antarctic Res. Ser. Vol 30:27-34.

Atlas, R.M., and R. Bartha. 1987. Microbial Ecology: Fundamentals and Applications. Benjamin-Cummings. Menlo Park, CA.

Baharaeen, S. and H. S. Vishniac. 1982. *Cryptococcus lupi* sp. nov., an Antarctic basidioblastomycete Int. J. Syst. Bacteriol. 32:229-232.

Balkwill, D.L. 1989. Numbers, diversity and morphological characteristics of aerobic, chemoheterotrophic bacteria in deep subsurface sediments from a site in South Carolina. Geomicrobiol J 7:33-52.

Balkwill, D.L., Fredrickson, J.K., and J.M. Thomas. 1989. Vertical and horizontal variations in the physiological diversity of the aerobic chemohetertrophic bacterial microflora in deep southeast coastal plain subsurface sediments. App. Envir. Microbiol.55(5): 1058-1065.

Banttari, E.E., and J.R. Venette. 1980. Aerosol spread of plant viruses: Potential role in disease outbreaks. Ann. New York Acad. Sci. 353: 167-173.

Bartholomew, J.W. and G. Paik. 1966. Isolation and identification of obligate thermophilic spore forming bacilli from ocean basin cores. J. Bacteriol. 92:635638.

Baylor, E.R., Baylor, M.B., Blanchard, D.C., Syzdek, L.D., and C. Appel. 1977. Virus transfer from surf to wind. Science 198: 575-580.

Beeman, R.E. and J.M. Suflita. 1990. Evaluation of deep subsurface sampling procedures using serendipitous microbial contaminants as tracer organisms. Geomicrobiol J 7:223-233.

Benson, L.V., J.W. Burdett, M. Kashgarian, S.P. Lund, F.M. Phillips and R.O. Rye. 1996. Climatic and hydrologic oscillations in the Owens Lake basin and adjacent Sierra Nevada, California. Science 274:746-749.

Bergh, O. Borsheim, G., Bratbak, G., and H. Heldal. 1989. High abundance of viruses found in aquatic environments. Nature (Lond). 340: 467-468.

Betancourt J.L, Van Devender T.R, and P.S. Martin (eds). 1990. Fossil Packrat Middens: The Last 40,000 Years of Biotic Change. Univ. Arizona Press, Tucson.

Betancourt J.L., Schuster W.S., Mitton J.B., and R.S. Anderson. 1991. Fossil and genetic history of a Pinyon Pine (*Pinus edulis*) isolate. Ecology 72:1685-1697.

Büttner, C., and F. Nienhaus. 1989. Virus contamination of soils in forest ecosystems of the Federal Republic of Germany. Eur. J. For. Pathol. 19: 47-53.
Cameron, R.E., Morelli, F.A. and R.M. Johnson. 1972. Bacterial species in soil and air of the Antarctic Continent. Antarc. J of the US 7(5):187-189.
Cameron, R.E., and F.A. Morelli. 1974. Viable microorganisms from ancient Ross Island and Taylor Valley drill core. Antarc. J. of the US 9:113-116.
Cano R.J., M.K. Borucki. 1995. Revival and identification of bacterial spores in 25- to 40-million-year-old Dominican amber. Science 268:1060-1064.
Cardot, J., Cummings, C. E., Evans, A. W., Peck, C. H., Saccardo, P.A., Saunders, DeA., Theriot, I. and W. Trelease. 1910. Cryptogamic Botany. vol v of Harriman Alaska Series publication 1994. Smithsonian Institution, Washington, D.C. 53 pp.
Castello, J.D., D.K. Lakshman, S.M. Tavantzis, S.O. Rogers, G.D. Bachand, R. Jagels, J. Carlisle, and Y. Liu. 1995. Detection of infectious tomato mosaic tobamovirus in fog and clouds. Phytopathology 85: 1409-1412.
Castello, J.D., S.O. Rogers, W.T. Starmer, C. Catranis, L. Ma, G. Bachand, Y. Zhao and J.E. Smith. 1999. Detection of tomato mosaic tobamovirus RNA in ancient glacial ice. Polar Biology 22:207-212.
Catranis, C., and W.T. Starmer. 1991. Microorganisms entrapped in glacial ice. Ant. J. of the U. S. 26: 234-236.
Cross, T., and R.W. Atwell. 1974 Recovery of viable Thermoactinomycete endospores from deep mud cores. In: Barker A. N., G. W. Gould, and J Wold (eds.) Spore Research, Academic Press, London. pp 11-20.
di Menna, M.D. 1966a. Three new yeasts from Antarctic soils: *Candida nivalis*, *Candida gelida* and *Candida frigida* spp. n. Antonie van Leeuwenhoek. 32:25-28.
di Menna, M.D. 1966b. Yeasts in Antarctic soils. Antonie van Leeuwenhoek. 32:29-38. Ekelof, W. 1908. Bacteriologische studien während der schsedischen Sudpolar expedtion, 1901-03. Stockholm, Sweden.
Dmitriev, V.V., D.A. Gilichinskii, R.N. Faizutdinova, I.N. Shershunov, W.I. Golubev, and V.I. Duda. 1997. Occurrence of viable yeasts in 3-million-year-old permafrost in Siberia. Mikrobiologiya 66:655-660.
Egorowa, A.A. 1931. Über Bakterien in fossilen Eis. Arktis 4:5-12.
Fell, J.W. 1974. Distribution of yeasts in water masses of the Southern Ocean. In: R. Colwell and R. Morita (eds.) Effect of the Ocean Environment on Microbial Activities. Univ. Park Press.
Fell, J.W. 1976. Yeasts in oceanic regions. In: Recent Advances in Aquatic Mycology. (Jones, E. B. G., ed). pp. 93-124, Elek Science, London.
Fell, J.W. and I.L. Hunter. 1974. *Torulopsis austromarina* sp. nov. A yeast isolated from the Antarctic Ocean. Antonie van Leeuwenhoek. 40:297-306.
Fell, J.W. and H. J. Phaff. 1967. Three new yeasts: *Cryptococcus dimennae*, *Cr. kutzingii* and *Cr. lactativorous* spp. Antonie van Leeuwenhoek, 33:467-472.
Fell, J.W., Statzell, A., Hunter, I.L., and H. J. Phaff. 1969. *Leucosporidium* gen. nov. The heterobasidomycetous stage of several yeasts of the genus *Candida*. Antonie van Leeuwenhoek. 35:433-462.
Fell, J.W., Hunter, I.L., and A.S. Tallman. 1973. Marine basidiomycetous yeasts (*Rhodosporidium* spp. n.) with tetrapolar and multiple allelic bipolar mating systems. Can J. Microbiol. 19: 643-657.
Fell, J. W. and S.Y. Newell. 1998. Biochemical and molecular methods for the study of marine fungi In: K. Cooksey (ed) Molecular Approaches to the Study of the Oceans Chapman & Hall, pp. 259-272.
Fell, J.W. and A.S. Tallman. 1982. Multiple allelic incompatibility factors among bifactorial strains of the yeast *Leucosporidium* (*Candida*) *scotti*i Curr.Microbiol. c7 213-216.
Fitt, B.D.L., McCartney, H.A., and P.J. Walkiate. 1989. The role of rain in dispersal of pathogen inoculum. Ann. Rev. Pathol. 27: 241-270.
Fliermans, C.B. and D.L. Balkwill. 1989. Microbial life in deep terrestrial subsurfaces. Bioscience 39(6):370-377.
Fränkel, C. 1886. Ueber den Bakteriengehalt des Eis. Z. Hyg. Inf. Krank. 1886:302-314.
Garrison, D.L., Sullivan, C.W. and S.F.Ackley. 1986. Sea ice microbial communities in antarctica. BioScience 36(4):243-250.
Gest, H. and J. Mandelstam. 1987. Longevity of microorganisms in natural environments. Microbiological Sciences 4:69-71.
Ghabrial, S.A. 1994. New developments in fungal virology. Adv. Virus Res. 43: 303-388.

Gloster, J., Sellers, R.F., and A.I.Donaldson. 1982. Long distance transport of foot-and-mouth disease virus over the sea. Veterinary Record 110: 47-52.
Goto, S., Sugiyama, J., and H. Iizuka. 1969. A taxonomic study of Antarctic yeasts. Mycologia 61:748-774.
Grant, R.H., Scheidt, A.B., and L.R.Rueff. 1994. Aerosol transmission of a viable virus affecting swine: Explanation of an epizootic of pseudorabies. Int. J. Biometeorol. 38: 33-39.
Jacobi, V., and J.D. Castello. 1991. Isolation of tomato mosaic virus from waters draining forest stands in New York State. Phytopathology 81: 1112-1117.
Johnson, R.M., Madden, J.M. andJ.R. Swafford. 1978. Taxonomy of Antarctic bacteria from soils and air primarily of the McMurdo Station and Victoria Land Dry Valleys Region. Ant. Res. Series 30:35-64 AGU Washington, D.C.
Karl, D.M., D.F. Bird, K. Björkman, T. Houlihan, R. Shackelford, and L. Tupas. 1999. Microorganisms in the accreted ice of Lake Vostok, Antarctica. Science 286:2144-2147.
Kegler, H., E. Fuchs, H.D. Knapp, and F. Ehrig. 1994. Untersuchungen zum vorkommen pflanzenpathogener viren im naturschutzgebiet insel Vilm (Biosphärenreservatsüdost-rügen). Arch. Phytopath. Pflanz. 29: 211-216.
Kobayasi, Y. 1982. Ecology and distribution of arctic lower fungi pp. 16-26.In: Laursen, G. and Ammirati, J. F. (eds.). Arctic and Alpine Mycology - The First International Symposium on Arcto-Alpine Mycology. Seattle; Univ. of Washington Press, 559 p.
Kobayasi, Y., Hiratsuka, N., Korf, R., Tubaki, K., Aoshima, K., Soneda, M. and J. Sugiyama. 1967. Mycological Studies of the Alaskan Artic. reprinted from the Annual Report of the Institute for Fermentation, Osaka, Japan. No. 3.138 p.
Koenig, R. 1986. Plant viruses in rivers and lakes. Adv. Virus Res. 31: 321-333.
Larsen, P. 1931. Fungi of Iceland. The Botany of Iceland. vol 2, part 3. Lavrenko, G. Ye. 1966. Algae of a lake near Novolazarevskaya station. Soviet Antarctic Expedition Information Bull. 6(1):63-66.
Lemke, P.A. (ed.).1979. Viruses and Plasmids in Fungi. Marcel Dekker Inc. New York. 653 p.
Lind, J. 1924a. Ascomycetes and Fungi Imperfecti. Report of the Sci. Res. of the Norwegian Exped. to Nov. Zeml. No. 19.
Lind, J. 1910a. Fungi collected in arctic North America by the Gjöa Exped. 1904-1906. Vid. Selsk. Skrifter I 1909 No. 9.
Lind, J. 1924b. Fungi collected on the north coast of Greenland by the late Dr. Th. Wulff. Medd. om Grønland Bd. 64:291-302.
Lind, J. 1910b. Fungi from north-east Greenland. Medd. om Gr0nland Bd. 43:149-162. Lind. J. 1927. The geographical distribution of some artic micromycetes. Kgl. Danske Videnskabernes Selskab Biologiske Neddelelser VI, nr. 5.
Lind, J. 1926. Micromycetes from north western Greenland. Medd. om Grønland Bd. 71: 161-179. Lind. J. 1934. Studies on the geographical distribution of arctic circumpolar micromycetes. Kgl. Danske Videnskabemes Selskab. Biologiske Meddelelser XI, nr. 2. 152 p.
Ling, H.U. and R.D.Seppelt. 1990. Snow algae of the Windmill Islands, continental Antarctica. *Mesotaenium berggrenii* (Zygnematales, Chlorophyta) the alga of grey snow. Antarct.Aci 2(2):143-148.
Ma, L.J., H. Fan, C. Catranis, S.O. Rogers, and W.T. Starmer. 1997. Isolation and characterization of fungi entrapped in glacial ice. Inoculum 48(3): MSA Abstracts, p. 23.
Ma, L., C. Catranis, W.T. Starmer, and S.O. Rogers. 1998. Study of glacial ice - a source of ancient fungi. Inoculum49(2):MSA Abstracts, p. 34.
Ma, L., C. Catranis, W.T. Starmer, and S.O. Rogers. 1999. Revival and characterization of fungi from ancient polar ice. Mycologist, 13:70-73.
Ma, L., S.O. Rogers, C. Catranis and W.T. Starmer. 2000. Detection and characterization of ancient fungi entrapped in glacial ice. Mycologia 92:286-295.
McKay, C.P. 1993. Relevance of Antarctic microbial ecosystems to exobiology. Ch. 15, pp. 593-601 In: Friedman, E. I. (ed.). Antarctic Microbiology. John Wiley & Sons, Inc. New York. 634 p.
Meschinelli, L. 1902. Fungoum fossilium hucusque cognitorum Icognographia. J. Calla, Venice.
Milligan, K.L.D., and E.M. Cosper. 1994. Isolation of virus capable of lysing the brown tide microalga, Aureococcus anophagefferens. Science 266: 805-807.

Noble, R.T., and J.A. Fuhrman. 1996. Virus decay and its causes in coastal waters. Appl. Environ. Microbiol. 63: 77-83.
Orlob, G., and P.R. Lorenz. 1968. Survival of tobacco mosaic virus in space. Phytopathology 58: 955-956.
Phillips, F.M., M.G. Zreda, L.V. Benson, M.A Plummer, D. Elmore and P. Sharma. 1996. Chronology for fluctuations in late Pleistocene Sierra Nevada glaciers and lakes. Science 274:749-751.
Pia, J. 1927. Thallophyten. In: Hirmer, M. (ed.). Handbuch der Paläobotanik. 1:31-136. Oldenbourg, Münchin & Berlin.
Pirozynski, K.A. 1976. Fossil Fungi. Annual Review of Phytopathology 14:237-246.
Poinar, G. D. Jr., and G.M. Thomas. 1984. A fossil entomogenous fungus from Dominican amber. Experientia 40:578-579.
Prescott, L.M., Harley, J.P., and D.A. Klein. 1996. Microbiology. 3rd Ed. W.C. Brown Publishers, Dubuque, IA. 935 p.
Priscu, J.C., E.E. Adams, W.B. Lyons, M.A. Voytek, D.W. Mogk, R.L. Brown, C.P. McKay, C.D. Takacs, K.A. Welch, C.F. Wolf, J.D. Kirchtein, and R. Avci. 1999. Geomicrobiology of subglacial ice above Lake Vostok, Antarctica . Science 286:2141-2144.
Proctor, L.M., and J.A. Fuhrman. 1990. Viral mortality of marine bacteria and cyanobacteria. Nature 343: 60-62.
Rao, V.C., and J.L. Melnick. 1986. Environmental Virology. Am. Soc. Microbiol., Washington DC, 88 p.
Riley, R.L., and F. O'Grady. 1961. Airborne Infection: Transmission and Control.MacMillan, New York. 180 p.
Rogers, S.O., L. Ma, J.D. Castello, Y. Zhao, J.E. Smith, and W.T. Starmer. 1999. Dilemmas and enigmas encased in ancient ice. XVI Internatioal Botanical Congress Abstracts, p. 452, no. 960.
Rostrup, E. 1888. Fungi Grøenlandiae. Medd. om Gønland Bd. 3:517-590.
Seaward M.R.D., Cross, T., and B.A.Unsworth. 1976. Viable bacterial spores recovered cfrom an archaeological excavation. Nature 261:407-408.
Service, R.F. 1997. Microbiologists explore life's rich, hidden kingdoms. Science 275: 1740-1742.
Seward, A.C. 1898. Fossil Plants. 1:205-222. Cambridge Univ. Press, Cambridge.
Shi, T., R.H. Reeves, D.A. Gilichinsky, and E.I. Friedman. 1997. Characterization of viable bacteria from Siberia permafrost by 16S rDNA sequencing. Microb. Ecol. 33:169-179.
Shuval, H.I., Guttman-Bass, N., Applebaum, J., and B. Fattal. 1989. Aerosolized enteric bacteria and viruses generated by spray irrigation of wastewater. Water Sci. Technol. 21: 131-135.
Sinclair, N.A., and J.L. Stokes. 1965. Psychrophilic yeasts from the polar regions. Can. J. Microbiol. 11:259-269.
Sneath P.H.A. 1962. Longevity of micro-organisms. Nature 195:643-646.
Spaulding W.G. 1985. Vegetation and climates of the last 45,000 years in the vicinity of the Nevada Test Site, south-central Nevada. U.S. Geological Survey Professional Paper 1329.
Spaulding W.G. 1990. Vegetation and climatic development of the Mojave Desert: The last glacial maximum to the present. In: JL Betancourt, TR Van Devender, PS Martin (eds), Packrat Middens: The last 40,000 years of biotic change, , University of Arizona Press, Tucson. pp166-199.
Spaulding W.G. 1991. A middle Holocene vegetation record from the Mojave Desert and its paleoclimatic significance. Quaternary Res. 35:427-437.
Spaulding W.G, Leopold E.B, and T.R. Van Devender. 1983. Late Wisconsin paleoecology of the American Southwest. In: SC Porter (ed), The Late Pleistocene. University of Minnesota Press, Minneapolis. pp.259-293.
Spaulding W.G, and L.J. Graumlich. 1986. The last pluvial climatic episodes in the deserts of southwerstern North America. Nature 320:441-444.
Staley, J.T., Irgens, R.L., and R.P. Herwig. 1989. Gas vacuolate bacteria from the sea ice of Antarctica. Appl. Env. Microbiol. 55: 1033-1036.
Stubblefield, S. P., and T. Taylor. 1988. Tansley Review No. 12 Recent advances in paleomycology. New Phytol. 108:3-25.
Sugiyama, J. 1969. Polar mycology in Antarctica II. The world's last frontier. Polar News 15:37-47.
Sun, S.H., M. Huppert, and R.E. Cameron. 1978. Identification of some fungi from soil and air of Antarctica. Paper 1 In: Parker, B. (ed.) Ant Res Ser 3:1-26.

Tiffney, B.H., and E.S. Barghoorn. 1974. The fossil record of the fungi. Occasional Papers of the Farlow Herbarium 7:1-42.
Vishniac, H.S. 1985. *Cryptococcus friedmannii*, a new yeast from the Antarctic. Mycologia 77:149-153.
Vishniac, H.S. and S. Baharaeen. 1982. Five new basidioblastomycetous yeast species segregated from *Cryptococcus vishniacii* emen. auct. an Antarctic yeast species comprising four new varieties. Int. J. Syst. Bacteriol. 32:437-445.
Vishniac, H.S. and H.P. Hampfling. 1979. *Cryptococcus vishniacii* sp. nov. an Antarctic yeast. Inter. J. Syst. Bacteriol. 29.153-158.
Vishniac, H.S. and S.E. Mainzer. 1973. Antarctica as a Mars model. Life. Sci. Space Res. 11:5-8.
Vreeland, R.H., W.H. Rosenzweig, and D.W. Powers. 2000. Isolation of a 250 million-year-old halotlerant bacterium from a primary salt crystal. Mature 407:897-900.
Wolf, F.A. and F.T. Wolf. 1947. The Fungi. vol. 2. John Wiley & Sons, New York.
Zaitlin, M., and H.W. Israel. 1975. Tobacco mosaic virus (type strain). Commonw. Mycol. Instit./Assoc. Appl. Biol. Descriptions of Plant Viruses, No. 151, 5 p.

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