Isolation and Identification of Bacteria from Ancient and Modern Ice Core Archives

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Abstract

Glacial ice traps and preserves soluble chemical species, gases and particulates including pollen grains, fungal spores and bacteria in chronologically-deposited archives. We have constructed an ice-core sampling system that melts ice only from the interior of cores, thereby avoiding surface contamination, and using this system we have isolated, cultured and characterized bacteria from ice cores that range from 5 to 20,000 years in age and that originating from both polar and non-polar regions. Low-latitude, high-altitude non-polar ice cores generally contain more culturable bacteria than polar ices, consistent with closer proximities to major biological ecosystems. Direct plating of melt-water from a 200-year old sample of ice from the Guliya ice cap on the Tibetan Plateau (China) generated ~180 bacterial colonies per ml [colony forming units/ml; (cfu/ml)], whereas melt water from late Holocene ice from Taylor Dome in Antarctica contained only 10 cfu/ml, and <10 cfu/ml were present ice of the same age from the Antarctic Peninsula and from Greenland. Based on their small-subunit ribosomal RNA-encoding DNA (rDNA) sequences many, but not all of the bacteria isolated are spore-forming species belong to *Bacillus* and *Actinomycete* genera. Non-chronological fluctuations are observed in the numbers of bacteria present, consistent with episodic deposition resulting from attachment to larger particulates.

Background

Snowfalls accumulate as glacial ice that traps gases, chemical species and particulates in a chronological record that can be accessed by ice-core sampling. Ice-cores collected from both polar and non-polar locations are archived at the Byrd Polar Research Center (BPRC) at the Ohio State University, and we are currently isolating bacteria from ice cores for which physical and chemical studies have already established ages and the climatic conditions prevailing when they were formed. The goals are to determine the numbers and types of bacteria recoverable from ice-cores from

different locations, how these parameters change in response to climate changes, and to obtain estimates of longevity of bacteria trapped in these frozen environments. With the recovery of ice from the Guliya ice cap on the Tibetan Plateau (China) that is >500,000 years old (Thompson et al., 1997), the opportunity exists to evaluate microbial survival in ice on a time scale that is meaningful for inter-planetary transport frozen within coments, and relevant to the idea that microbial life may continue to exist frozen below the surface of Mars, or within the ice or sub-ice ocean on Europa.

Previous reports have described the isolation of bacteria and fungi from glacial ice (Abyzov, 1993; Dancer *et al*. 1997) and permafrost (Gilichinsky *et al*. 1993; Shi *et al*. 1997), with many isolates being species that differentiate naturally into radiation and desiccation-resistant spores or cysts. However, these are mostly reports of single sampling experiments whereas our goal is to compare the numbers and types of bacteria that can be recovered from ice of different ages from the same location, and from different ice cores of the same age from different global locations. Here we report the results of bacterial isolations from ice cores from Greenland, China, Bolivia and Antarctica, that range from 5 and 20,000 years in age.

Sampling Technology

The exterior surface of an ice core is almost inevitably contaminated during drilling and subsequent transport back to the BPRC. An ice core sampling system has therefore been developed and constructed to melt and collect melt-water samples only from the "non-handled" interior of an ice core (Figure 1). A slice is cut from the end of the core, and the cut surface is disinfected by soaking in 95% ethanol for 2 minutes at -20^{o}C which dissolves away an additional layer of ice. Control experiments in which bacteria were swabbed intentionally onto the cut surface and onto the saw blade used to cut the ice core confirmed that such an ethanol wash effectively eliminated surface contamination. The core is then positioned vertically in the sampling device with the cut and disinfected end contacting the sampling head. By using different sized sampling heads, ice can be

dissolved from one-half or one-quarter of the core facilitating repetition of the experiment, i.e., bacteria can be isolated from more than one sample of melt water of the same age from the same ice core. The funnel-shaped sampling head is heated internally, heat from the sampling head melts the contacted ice and the melt water generated is collected through a central port at the base of the funnel. The heated sampling head moves vertically upward through the interior of the ice core, generating and collecting melt water only from the interior of the core. By using a fraction collector, the melt water from increasing heights within the core can be collected sequentially as separate samples.

Evaluation of Bacterial Growth Media and Growth Conditions

Plating samples of melt water on agar-solidified, commercial growth media containing low levels of nutrients, such as *Actinomycetes* isolation agar and R2A (Difco), or on nutrient agar (Bacto) diluted 100-fold more than normally recommended results in the growth of more bacterial colonies that plating on full-strength rich nutrient media. Such nutritionally restricted conditions reduce the tendency of otherwise fast-growing species to dominate in bacterial isolations from environmental samples and apparently also remove an "immediate-growth" stress which increases the survival and recovery of cells that have accumulated cellular damage during long periods on frozen inactivity. Some bacterial colonies appear only after 1-2 months incubation at 25^{o}C, and some have appeared first after ~4 months incubation at 10^{o}C. Nevertheless, on subsequent sub-culture, most isolates form visible colonies after only 2-3 days incubation at 25^{o}C. All the isolates so far characterized have been isolated under aerobic growth conditions, but isolations under anaerobic growth conditions are currently in progress.

Initially each isolate is characterized phenotypically for colony color and morphology, by light microscopy, growth temperature range, growth on different media and resistance/sensitivity to antibiotics. The 16S rDNA sequences corresponding to nucleotides 515 to 1492 of *Escherichia coli* 16S rDNA are then determined for all isolates exhibiting phenotypic differences. Based on such

sequences, each isolate is identified in terms of its nearest, previously-characterized relative (Maidak *et al*, 1999; see Table 1). Although most phenotypically different isolates have different 16S rDNA sequences, some with different pigments that form colonies with different morphologies have been found to have identical 16S rDNA sequences.

Geographic Differences in the Numbers and Diversity of Bacterial Isolates

When aliquots of melt water from ice cores of different ages from Greenland, China, Bolivia and Antarctica were plated as described above, no colonies grew on plates inoculated with melt water from 150-year old Antarctic Peninsula ice or from 1500-year old Sajama ice (Bolivia), but ~180 bacterial cfu/ml were present in the melt water from 200-year old Guliya ice (China). Melt water from Late Holocene (~1,800 years old) polar ice from Taylor Dome (Antarctica) contained ~10 cfu/ml, and whereas Late Holocene ice from the Antarctic Peninsula and Greenland (Summit and Dye 2) contained >2 cfu/ml. In contrast to the 1500-year old ice, aliquots of melt water derived from modern and from 12,000 to 20,000-year old Sajama ice (Bolivia) contained 5-20 cfu/ml. Regardless of their geographic origin, many of the isolates formed highly pigmented colonies, consistent with pigments providing a survival advantage, presumably by increasing protection against solar irradiation during airborne distribution and exposure on the surface of a glacier.

Most of the isolates have 16S rDNA sequences closely related to non-sporulating Gram-positive bacteria, or to Gram-positive spore-forming *Bacillus* or *Actinomycetes* species. However, overall, bacteria that belong to *Acinetobacter, Arthrobacter, Aureobacterium, Bacillus, Brevibacterium, Cellulomonas, Clavibacter, Flavobacterium, Friedmanniella, Kurthia, Listeria, Microbacterium, Micrococcus, Micromonospora, Mycobacterium, Nocardioides, Paenibacillus, Propioniferax, Sphingomonas, Staphylococcus* and *Stenotrophomonas* genera have been isolated based on their having 16S rDNA sequences that are 95% identical to that of a previously characterized species (Table 1) . Isolates from both 5- and 200-year old Guliya ice have 16S rDNA sequences that are >99% identical to the 16S rDNA sequences of *Bacillus subtilis, Arthrobacter*

agilis or *Clavibacter michiganensis* and, interestingly, an almost identical *C. michiganensis*-related isolate was obtained from 12,000-year old Sajama ice.

Plating melt water also results in the growth of fungal colonies, consistent with the widespread airborne distribution of fungal spores, but attempts have not so far been made to identify the fungal species.

Conclusions

Bacteria revived from ice cores have probably have endured desiccation and solar irradiation during airborne transport, followed by freezing, a period of frozen dormancy, and thawing and we are currently determining if our ice-core isolates have structural and/or biochemical features in common that can be related to their ability to survive such environmental abuse. Almost certainly, most such cells will have sustained some cellular damage, and the long incubation times required for initial colony formation are consistent with the idea these bacteria require a substantial period of time to repair accumulated cell damage before they can grow and divide successfully (Dodd *et al.* 1997).

Most melt water samples from ice cores from low-latitude, high-altitude glaciers in the Andes and Himalayas have been found to contain more culturable bacteria than samples from polar ices, consistent with these non-polar glaciers being closer to large biological ecosystems. Among the polar ices, the highest numbers of bacteria (~10 cfu/ml) have been recovered from Taylor Dome ice, from a site located at the head of the Taylor Valley in the Dry Valley complex of Antarctica. This is a very dry and very cold environment but there is substantial cryptoendolithic growth of algae, fungi and bacteria within the sandstone that dominates this region (Boyd 1967; Cameron 1971). Changes in the numbers of bacteria present within non-polar ice appear to be related to changes in climate. For example, in an earlier cooler, wetter period in S. America, the abundance of local vegetation increased and presumably therefore the amounts of airborne particulates will have also increased. Particulates transport bacteria, and the result is an increased number of bacteria in Andean glacial ice formed at that time.

Most of the bacteria isolated to date are related to ubiquitous soil inhabitants, to species that have been isolated frequently in previous microbiological surveys of environmental samples from around the world. Many are species that differentiate into spores or cysts, cell types that tolerate environmental stress and survive extended periods of dormancy. For example, bacterial endospores remain viable for thousands of years (Abyzov 1993), and possibly even for millions of years in amber (Cano *et al*. 1995), although this has been disputed (Priest 1995). Intriguingly, however, some of our isolates are closely related to species that were previously only isolated from frozen tundra soil, glacial ice, polar sea ice, or from the Antarctic Dry Valley region (Gosink and Staley 1995; Bowman *et al*. 1997; Schumann *et al*. 1997; Shi *et al*. 1997; Zhou *et al* 1997; Junge *et al*. 1998; Siebert and Hirsch 1988). The isolation of these species from geographically diverse but always cold and often frozen environments, suggests that they may have evolved cell structures and metabolic lifestyles specifically to grow and survive under these conditions. Identifying such cold-survival mechanisms would be inherently interesting, but would also provide important data for predicting the microbiology that might exist frozen in non-terrestrial environments.

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Table 1. Bacteria isolated from non-polar glacial ice.

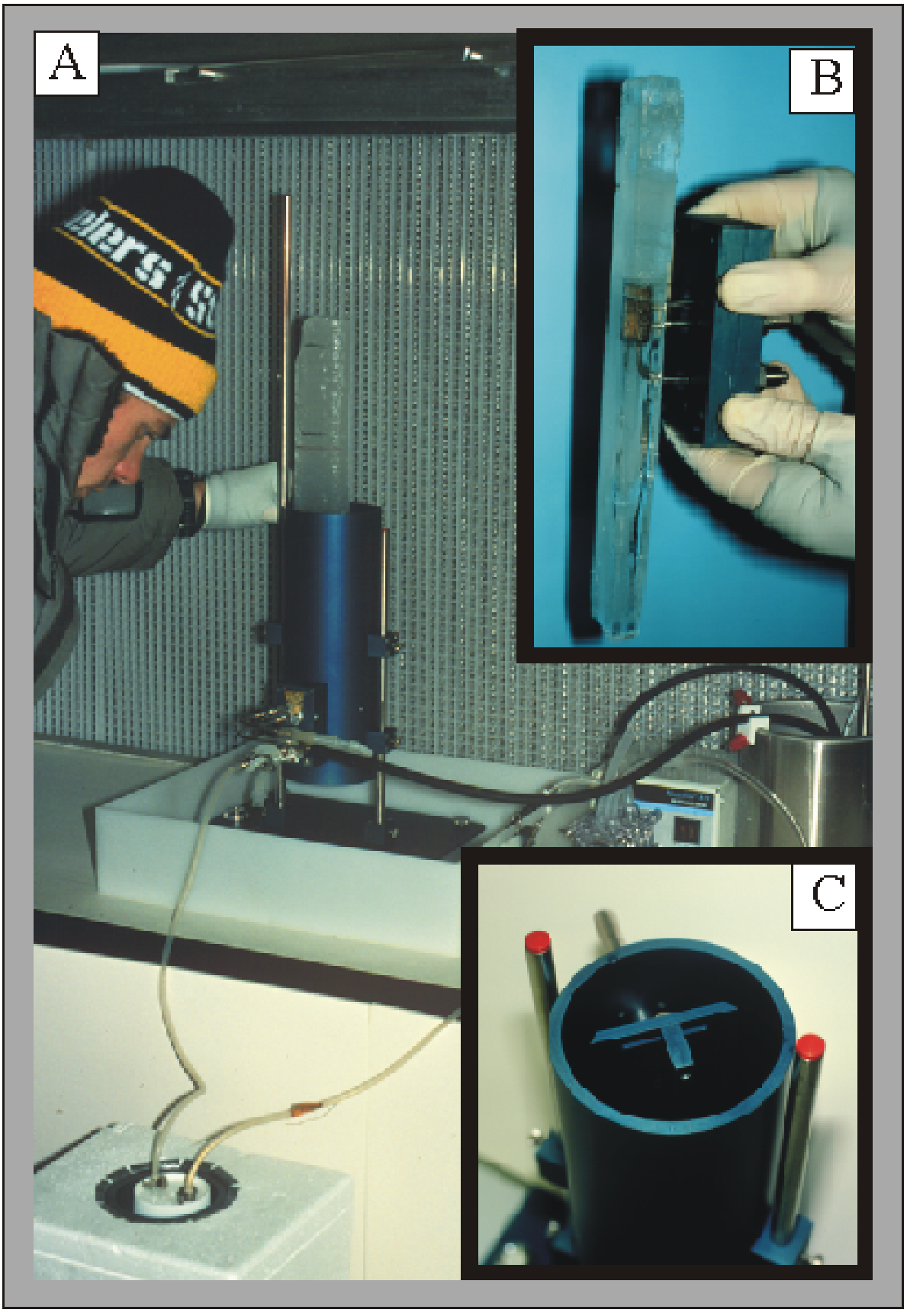
Nearest Phylogenetic Neighbor†	Source and Age of Ice‡
Acinetobacter radioresistans	G5
Arthrobacter agilis	G5, G200
Arthrobacter globiformis	G200
Aureobacterium liquefaciens	G5
Aureobacterium testaceum	SB12K
Bacillus subtilis	G5, G200
Bacillus thuringiensis	G5, SB12K
Bacillus firmus	G200
Bacillus sporothermodurans	G200
Bacillus psychrophilis	SB100
Bacillus sp.10	G200
Brevibacterium acetylicum	SB12K
Cellulomonas turbata	G200
Cellulomonas hominis	SB12K
Clavibacter michiganensis	G5, G200, SB12K
Flavobacterium okeanokoites	G5
Friendmanniella antarctica	SB12K
Microbacterium aurum	G200
Microbacterium lacticum	SB12K
Micromonospora purpurea	G200
Micrococcus lylae	SB20K
Mycobacteria komossnese	SB12K
Norcardia corynebacteroides	SB12K
Norcardioides plantarum	SB12K
Paenibacillus amylolyticus	G5
Paenibacillus polymyxa	G5
Paenibacillus lautus	G200
Planococcus kocuri	SB150
Propioniferax innocua	G200
Staphylococcus aureus	G5
Stenotrophomonas africae	G5

†Based on 16S rDNA sequences being >95% identical to that of the named species, determined by the ShowDistance function of PAUP 4.0, beta version (Maidak *et al.* 1999).

‡ G5 and G200 designate 5 and 200-year old ice, respectively, from Guliya (Bolivia). SB100, SB150, SB12K and SB20K designate 100, 150, 12,000 and 20,000- year old ice, respectively, from Sajama (China).

Figure Legend

Figure 1. Ice core sampler. A. The complete sampler with an ice core inserted in the ice-melting unit. All components of the system are sterilized by autoclaving and then assembled inside a laminar air-flow hood housed within an a -20°C walk-in freezer. B. The sampling head after movement through a core and removal of a cylindrical section from inside the core. C. Moveable dividers that facilitate melting through half or quarter sections of the core.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