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Research Paper

Subfreezing Activity of Microorganisms and the Potential Habitability of Mars' Polar Regions

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ABSTRACT

The availability of water-ice at the surface in the Mars polar cap and within the top meter of the high-latitude regolith raises the question of whether liquid water can exist there under some circumstances and possibly support the existence of biota. We examine the minimum temperatures at which liquid water can exist at ice grain–dust grain and ice grain–ice grain contacts, the minimum subfreezing temperatures at which terrestrial organisms can grow or multiply, and the maximum temperatures that can occur in martian high-latitude and polar regions, to see if there is overlap. Liquid water can exist at grain contacts above about −20°C. Measurements of growth in organisms isolated from Siberian permafrost indicate growth at −10°C and metabolism at −20°C. Mars polar and high-latitude temperatures rise above −20°C at obliquities greater than ~40°, and under some conditions rise above 0°C. Thus, the environment in the Mars polar regions has overlapped habitable conditions within relatively recent epochs, and Mars appears to be on the edge of being habitable at present. The easy accessibility of the polar surface layer relative to the deep subsurface make these viable locations to search for evidence of life. Key Words: Mars polar regions—Liquid water—Habitable conditions—Subfreezing temperatures—Microorganisms. Astrobiology 3, 343–350.

INTRODUCTION

THE MARTIAN SUBSURFACE today is generally thought to meet the environmental requirements necessary to support life. Liquid water likely is the limiting factor (e.g., Jakosky, 1998) and can occur at depths of hundreds of meters to kilometers, where temperatures are warmer than at the surface (Carr, 1996; Malin and Edgett, 2000; Mellon and Phillips, 2001). Geochemical energy is available there through reactions of the water with the surrounding rock or by mixing in hydrothermal systems, and terrestrial organisms, at least, are able to take advantage of these sources of energy to support metabolism (Shock, 1997; Jakosky and Shock, 1998; Varnes *et al.*, 2003). While the deep subsurface is, therefore, a likely place to find life, it is difficult to access for

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exploration. We examine whether surface temperatures in the polar regions might allow the presence of liquid water and be able to support microorganisms during periods of increased tilt of the polar axis. The tilt, or obliquity, varies on timescales longer than 10^5 years and can reach values as high as about 60° (Ward, 1992; Laskar and Robutel, 1993; Touma and Wisdom, 1993). At these high values, solar heating of the polar regions increases, and surface and near-surface temperatures rise substantially.

To determine whether Mars' polar conditions at high obliquity overlap those in which life could exist, we consider the following: (i) the minimum temperatures at which thin films of liquid water can exist in soil; (ii) the minimum temperatures at which microorganisms can either grow or metabolize; (iii) the range of obliquity values that can occur, and the surface and subsurface temperatures that result; (iv) the distribution of polar ice and ground ice that could provide a source of melt water; and (v) the ability of microorganisms to survive and grow in such an environment. We draw on prior analyses in each area, along with new measurements that show the abundance of liquid water in soils at subzero temperatures and the growth and metabolism of microorganisms at temperatures as low as −20°C.

MINIMUM TEMPERATURE FOR LIQUID WATER

What is the minimum temperature at which liquid water can exist in ice or soil? Bulk water ice melts at 0°C. Dissolved salts lower the melting temperature, but there is little evidence that martian soils contain the quantities of salt necessary to produce more than a few degrees' melting-point depression (Clark and Van Hart, 1981). However, thin films of water can exist at subzero temperatures when grains of ice are in contact with each other or with individual soil grains. Liquid at grain contacts becomes stable as a result of the change in the energy state of the molecules because of the proximity of the grain boundaries (Neresova and Tsytovitch, 1966; Low *et al.*, 1968; Anderson and Tice, 1973; Frolov and Gusev, 1973; Price, 2000).

Figure 1 shows the amount of liquid water in a soil at subzero temperatures. These measurements were obtained using a water-content reflectometry probe that measures the period of an electromagnetic wave propagated through the soil; the wave period is dependent on the dielectric constant and, hence, liquid water content of the soil and is not sensitive to solid ice. This particular soil is a loam cryocept consisting of roughly 40% clay and 60% silt, sampled from alpine tundra at the Niwot Ridge Long Term Ecological Research site in the Colorado Front Range (Ley *et al.*, 2001, 2003). The minimum water content occurs at temperatures below about −20°C and represents unfrozen adsorbed water. Above −20°C, water in abundances greater than the amount of adsorbed water exists as liquid water and is present as thin films. At these low temperatures, there is a smooth gradation or transition between a thin film of liquid water and multiple layers of adsorbed water.

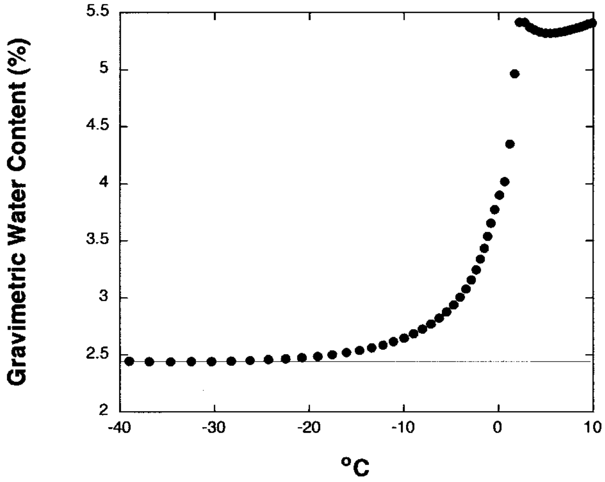

FIG. 1. Liquid water content of a loam soil as a function of temperature (see text). Measurements were made with an electromagnetic wave reflectometry probe, calibrated empirically over a temperature range of −40 to +10°C. Homogenized soil was placed in a steel cylindrical tube (10 cm diameter × 40 cm length) with the water content and thermistor probes inserted midway. The soil tubes were frozen with liquid nitrogen and allowed to warm slowly over a period of 2 days. Wave period and the temperature were recorded every 10 min.

Can these thin films be sufficiently thick down to −20°C to support biota? The films would have to be capable of physically containing organisms and of carrying out the necessary functions of transporting dissolved ions and allowing diffusion of both nutrients and waste products. Available evidence suggests that even adsorbed water in soils supports diffusion of ions (Anderson, 1967; Cary and Mayland, 1972; Ugolini and Anderson, 1973). The variability of the thickness of the thin films of water with location on the individual grains suggests that there will be places where organisms can be physically contained within the liquid; the physical presence of organisms also will affect the molecular energy levels of the water molecules and allow for their residing within the soil. In ice, channels and thin films of liquid water that contain impurities can exist at the boundaries between individual ice grains at temperatures as low as −20°C (Anderson, 1967; Mader, 1992a,b; Price, 2000). At temperatures below −20°C, it is not clear that liquid water would be present in sufficient amounts in either soils or ice to physically allow the presence of organisms.

MINIMUM TEMPERATURE FOR GROWTH OR METABOLISM

Organisms can metabolize at these temperatures as well. There is compelling evidence that microorganisms can take up nutrients and multiply at −10°C (Bakermans *et al.*, 2003). These bacteria survive in the laboratory in brines in which dissolved salts lower the freezing temperature. The presence of salts is not a requirement for growth at this low temperature, as these bacteria also grow when glycerol is used to depress the freezing point. In addition, ordering effects, surface charge, and polarity at cell membranes, rather than bulk liquid effects, may be equally important for adaptation to low temperatures (Gilichinsky *et al.*, 1993; Soina *et al.*, 1995; Mindock *et al.*, 2001); it is likely that organisms can function at these temperatures as long as liquid water is available. In addition, there is evidence both for mobility of ions in soils and for the uptake of nutrients by organisms down to −15°C (Priscu *et al.*, 1998; Carpenter *et al.*, 2000; Rivkina *et al.*, 2000; Bakermans *et al.*, 2003). Photosynthesis of Arctic and Antarctic lichens also has been measured at −10°C to −17°C by CO_2 exchange (Schroeter *et al.*, 1994; Kappen *et al.*, 1996; Lang, 1996).

Specific growth rates were obtained at temperatures from +16 to −10°C for five phylogenetically diverse psychrotolerant bacteria (Fig. 2A) that were previously isolated and described (Bakermans *et al.*, 2003). The temperature dependence of their growth rates is fit well by the Boltzmann–Arrhenius equation $\mu = Ae(-E/kT)$, where μ is the specific growth rate, A is a constant, E is an energy difference, k is Boltzmann's constant, and T is temperature in Kelvins. Cell growth was monitored by measuring the turbidity of cultures at 600 nm, and was confirmed at −10°C by monitoring plate counts. Extrapolation to −20°C yields a specific growth rate of 0.19 divisions/year (i.e., a generation time of 3.6 years). Growth rates this low would be very difficult to verify in the laboratory, much less *in situ*. Metabolic activity was also measured (and is reported here) for the same species at temperatures as low as −20°C, the coldest temperature for which metabolism has been confirmed to date; again, the rates are fit well by the Boltzmann–Arrhenius

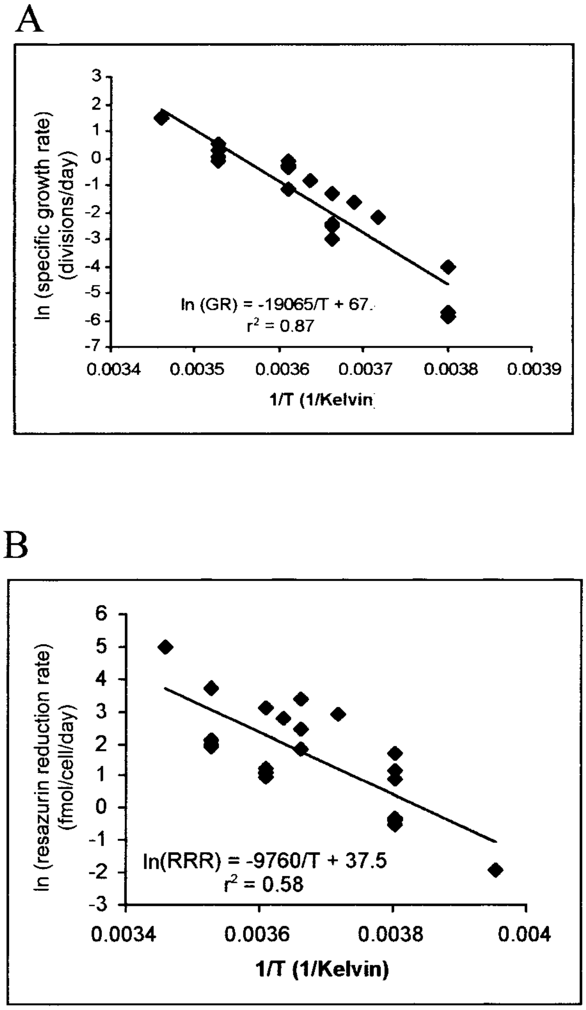

FIG. 2. Measurements of specific growth rate (A) and resazurin reduction rate (B) for five psychrotolerant eubacteria isolated from Siberian permafrost, shown as a function of inverse temperature. Best-fit line of the Boltzmann–Arrhenius equation is shown in both cases. Growth rates were determined to −10°C and reduction rates to −20°C.

equation (Fig. 2B). Metabolic activity was measured by a respiration assay based on the reduction of resazurin by dehydrogenases that are coupled to the electron transport system (Bakermans *et al.*, 2003). These data demonstrate the ability of bacteria to metabolize (but not necessarily reproduce) at −20°C.

Microorganisms may survive at these lowest temperatures by maintaining a minimal metabolism sufficient only for repair and maintenance of cell structures. Recent work suggests that cells begin to put energy preferentially into repair and maintenance rather than division and biosynthesis starting at ~4°C (Bakermans *et al.*, 2003). The cost of maintenance may eventually become so high that cell reproduction is prohibited at lower temperatures (Friedmann *et al.*, 1993; Karl *et al.*, 1999). Survival by utilizing energy for maintenance without reproducing may illustrate success measured by long life, rather than by the number of offspring (i.e., competitors) produced. Such an approach would argue that developing the proper regulatory strategy is key to the long-term survival of organisms as they approach the lower limits of their growth. We suspect that the lowest temperature at which terrestrial (and presumably martian) life can function probably is near −20°C.

MARS POLAR AND HIGH-LATITUDE TEMPERATURES

On Mars, temperatures at equatorial and midlatitudes regularly rise above −20°C and even 0°C (e.g., Kieffer *et al.*, 1977). The likely absence of water ice in these regions due to the high potential for sublimation into the atmosphere (Ingersoll, 1974; Farmer and Doms, 1979; Mellon and Jakosky, 1993, 1995), however, makes the presence of liquid water problematic. The presence of water ice at the surface on the polar cap (Farmer *et al.*, 1976; Kieffer *et al.*, 1976) and in the high-latitude regolith (Leighton and Murray, 1966; Mellon and Jakosky, 1993, 1995; Boynton *et al.*, 2002; Feldman *et al.*, 2002) points to these regions as places that have the potential for liquid water to exist.

We examine whether polar surface temperatures can rise to −20°C or above during periods of high obliquity. The present-day obliquity of Mars is 25.2°, and peak summertime polar-ice temperatures are ~205 K, or −68°C (Kieffer and Zent, 1992). The obliquity varies by up to 10° with periods of $\sim10^5$ and 10^6 years, because of gravitational forcing primarily from Jupiter (Ward, 1992). In addition, the "guiding center" of the obliquity varies with time because of resonant forcing and chaotic wandering. The timescale at which the obliquity becomes unpredictable is $\sim10^7$ years (Laskar and Robutel, 1993; Touma and Wisdom, 1993), meaning that the values cannot be known accurately for ages substantially greater than this. The obliquity can vary between extreme values of ~0° and 60°, and may have taken any value in this range prior to a few times the chaotic timescale (Laskar and Robutel, 1993).

We use simple thermal models to calculate what the polar temperatures would have been at high obliquity (Jakosky *et al.*, 1993, 1995). The key physical parameters that describe the system are taken from spacecraft measurements at the present epoch (Paige and Ingersoll, 1985; Kieffer, 1990). Figure 3A shows the resulting peak summertime temperatures. The spread in values at a given obliquity reflects the influence of the orbital eccentricity and argument of perihelion. The temperatures at the higher obliquities (shown by the open circles) include the latent heat of sublimation of water ice in the energy balance calculations; high sublimation rates result in a depression or a flattening of the curve. Notice that the peak temperatures at high obliquities are ~250 K, or −23°C, still slightly below the minimum temperature required to support life.

Other processes can increase this peak temperature, however. At high obliquity, CO_2 gas may desorb from the regolith because of the increased temperatures at high latitudes, and could as much as double the current atmospheric pressure of 6 mbar (Kieffer and Zent, 1992). This would result in an increased greenhouse warming that would raise surface temperatures by 3–5 K (Kasting, 1991). If sunlight penetrates into the polar ice and is absorbed below the surface, subsurface temperatures will rise to values higher than the surface temperatures. Radiative transfer calculations by Clow (1987) suggest that the depth of maximum heating, similar to the effective depth of penetration of sunlight, can be within the range of a few centimeters to a few tens of centimeters, depending on ice grain size and degree of contamination by dust. If sunlight penetrates ~5 cm on average, for example, then temperatures at this depth could be raised by ~5 K. Finally, if sublimation of substantial quantities

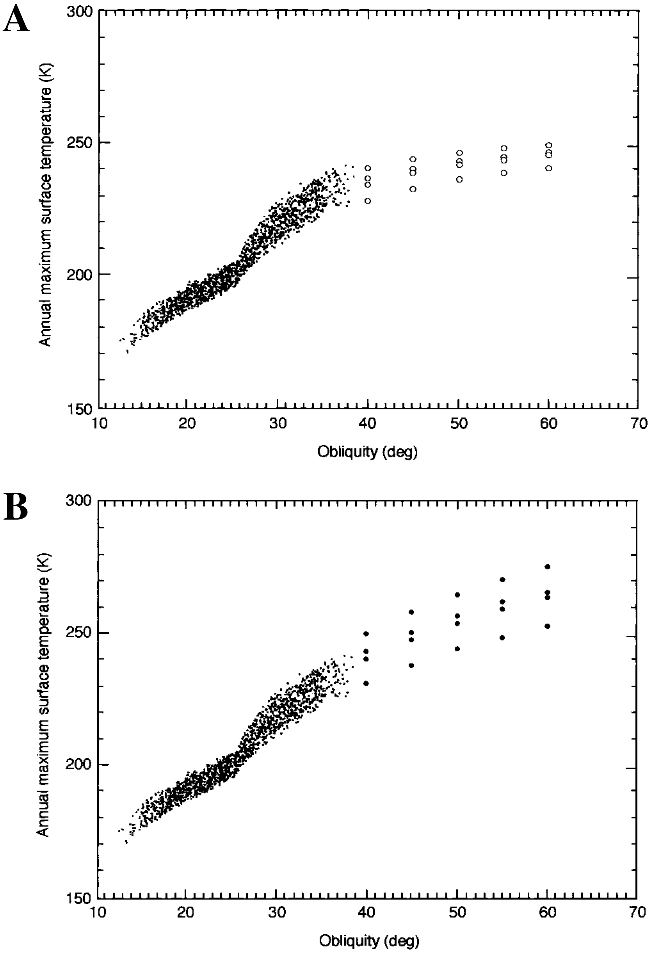

FIG. 3. Peak Mars summertime temperatures calculated as a function of obliquity using a simple thermal model that assumes present-day properties (see Jakosky *et al.*, 1993, 1995, for details of the models). A: Temperatures were calculated including energy effects of latent heat of water-ice sublimation, depressing temperatures at the highest obliquities. **B:** Temperatures were calculated without latent heat effects, appropriate for when sublimation is inhibited by a lag deposit of dust.

of water ice at high obliquity leaves behind a residual layer of dust, the dust would be darker than the ice (and would absorb more sunlight) and would act as a diffusive barrier to inhibit water-ice sublimation (Toon *et al.*, 1980; Hofstadter and Murray, 1990). The effects of inhibiting sublimation are shown in Fig. 3B, in which the latent heat of sublimation is not included in the surface energy balance. In this case, peak temperatures are >0°C at the highest obliquities, and temperatures above −20°C occur for an obliquity as low as ~45° (Costard *et al.*, 2002). The decrease in albedo in going to a dust-covered surface typically would raise temperatures by another ~20 K, and could allow temperatures above −20°C at obliquities below 35°.

Extrapolation to epochs of high obliquity is uncertain, because of lack of knowledge of how the pertinent physical parameters might change. It is plausible, however, that surface and subsurface temperatures on the polar ice can rise at least to the −20°C values required to support liquid water and active metabolism of microorganisms. If salts are present in even small amounts or if temperatures rise to higher values, the amount of liquid would be enhanced. The survival of organisms in terrestrial ice at subfreezing temperatures (Cameron and Morelli, 1974; Paerl and Priscu, 1998; Priscu *et al.*, 1998) supports the concept of organisms being able to exist in this type of environment.

What is the availability of water ice at high latitudes but off of the polar ice cap? Water vapor can diffuse from the atmosphere into the regolith and condense out as ice; water ice becomes stable within the top meter of the surface at latitudes poleward of about ±50° (Leighton and Murray, 1966; Mellon and Jakosky, 1993, 1995). γ-Ray and neutron measurements from the Mars Odyssey spacecraft show that the regolith at these latitudes, in fact, contains substantial quantities of water ice (Boynton *et al.*, 2002; Feldman *et al.*, 2002). The boundary between ice-free and ice-filled regolith should occur at a depth of ~10–100 cm, depending on latitude and surface thermophysical properties, where the annual average pore space water vapor density is below that of the atmospheric water vapor (Mellon and Jakosky, 1995). The ice distribution in the regolith thus is governed by the atmospheric water vapor, which in turn is controlled by the polar cap summertime temperatures; the ground-ice frost-point temperature will be very near to the peak summertime polar ice surface temperature (Mellon and Phillips, 2001). Thus, ground-ice in the high-latitude regolith at high obliquity will be at temperatures very near to or above the −20°C value at which thin films of liquid water and organisms can exist. (Note that, because of the dependence of this temperature on the amount of polar-cap water-ice sublimation, the substantially higher temperatures that can occur in the polar ice under some conditions may not translate into similarly higher ground-ice temperatures. Thus, the peak temperature here may not be very far above −20°C.)

DISCUSSION AND IMPLICATIONS

If organisms can occasionally metabolize and grow in the polar ice and high-latitude ground-ice, can they survive the colder periods that prevail at lower obliquity between the more clement epochs? Terrestrial microorganisms are routinely stored at −80°C without detrimental effects, and should survive as long as ice crystals do not disrupt cell membranes and accumulated radiation damage is not so severe that it cannot be repaired upon warming. The terrestrial evidence suggests that organisms can survive long periods in a dormant state, perhaps up to tens to hundreds of millions of years (Kennedy *et al.*, 1994; Cano and Borucki, 1995; Vreeland *et al.*, 2000; see, however, Wayne *et al.*, 1999). The very low martian temperatures at low and moderate obliquity would preclude the existence of liquid water that would most encourage degradation (Lindahl, 1993; Wayne *et al.*, 1999).

The presence of gullies and seeps at high latitudes that involve the release of water to the surface (Malin and Edgett, 2000) further suggests that liquid water can exist episodically. Whether the source of water is the subsurface (Mellon and Phillips, 2001) or nearer to the surface (Costard *et al.*, 2002; Christensen, 2003), it allows the opportunity for organisms to revive and repair themselves every few million years, even at very low temperatures that would allow metabolism but not necessarily growth; organisms could effectively reset any damaged systems (amino acid chirality, membrane permeability, damaged DNA, etc.), and thus allow very-long-term survival.

Thus, Mars today appears to be right at the edge of being habitable by microorganisms. An increase in obliquity to high values would result in temperatures that would allow sufficient quantities of liquid water to exist at the surface or within the top meter on the polar cap and at high latitudes to sustain microorganisms. While life might have a more difficult time surviving in these regions compared with deep-subsurface habitats, the polar regions are much more accessible. A spacecraft mission in search of dormant organisms, spores, or organic detritus from prior epochs, or geochemical evidence for prior liquid water having been present, could be directed toward a geographically distributed region rather than a specific location determined by the local geology. Also, rather than having to drill down hundreds of meters or more to access regions where liquid water had been present, access to the topmost meter likely would suffice. Both of these scenarios are within our present-day technological abilities.

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