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Terrestrial models for extraterrestrial life: methanogens and halophiles at Martian temperatures

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Abstract: Cold environments are common throughout the Galaxy. We are conducting a series of experiments designed to probe the low-temperature limits for growth in selected methanogenic and halophilic Archaea. This paper presents initial results for two mesophiles, a methanogen, *Methanosarcina acetivorans*, and a halophile, *Halobacterium* sp. NRC-1, and for two Antarctic cold-adapted Archaea, a methanogen, *Methanococcoides burtonii*, and a halophile, *Halorubrum lacusprofundi*. Neither mesophile is active at temperatures below 5 °C, but both cold-adapted microorganisms show significant growth at sub-zero temperatures (−2 °C and −1 °C, respectively), extending previous low-temperature limits for both species by 4–5 °C. At low temperatures, both *H. lacusprofundi* and *M. burtonii* form multicellular aggregates, which appear to be embedded in extracellular polymeric substances. This is the first detection of this phenomenon in Antarctic species of Archaea at cold temperatures. The low-temperature limits for both psychrophilic species fall within the temperature range experienced on present-day Mars and could permit survival and growth, particularly in sub-surface environments. We also discuss the results of our experiments in the context of known exoplanet systems, several of which include planets that intersect the Habitable Zone. In most cases, those planets follow orbits with significant eccentricity, leading to substantial temperature excursions. However, a handful of the known gas giant exoplanets could potentially harbour habitable terrestrial moons.
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Introduction

The past decade has seen the discovery of planetary companions of more than 100 stellar neighbours of the Sun. These planets are subject to a wide range of environments, from hot Jupiter-like planets orbiting within 0.05 AU of the central star at ambient temperatures (T_{amb}) exceeding 1000 °C to gas giants in Jupiter-like orbits at $T_{amb} \sim -150$ °C. A key issue facing astrobiology is assessing what subset of these environments could be hospitable to life; what are the upper and lower temperature limits for species growth and survival? The low-temperature limit for life is particularly important since, in both the Solar System and the Galaxy as a whole, cold environments are much more common than hot environments.

Mars and Europa are the prime Solar System targets in the search for life beyond Earth. Both present relatively hostile environments for mesophilic terrestrial life, but both seem capable of supporting liquid water, which is generally regarded as a prerequisite for life. In Europa's case, water, if present, resides under a thick ice cap, heated by the dissipation of tidal forces (Reynolds *et al*. 1987; Pappalardo *et al*. 1999). Mars, however, may be capable of maintaining liquid water on or near the surface. Recent results from the Neutron Spectrometer on Mars Odyssey indicate the presence of extensive deposits of water ice, particularly at latitudes above $\pm 50^{o}$ (Feldman *et al*. 2004). Measurements by the Pathfinder Lander and by the Mars Global Surveyor indicate that atmospheric pressures between ~6.9 and ~8.3 mbar, while the Viking Landers measured pressures are between 7 and

10 mbar (see Fig. 1 in Kuznetz & Gan 2002); all these values lie above the triple point of water (6.1 mbar). Moreover, surface temperatures can exceed freezing for several hours each day at higher latitudes in the 'summer' hemisphere (see Fig. 2 in Kuznetz & Gan 2002), although there is only a limited window for liquid water as it evaporates at $\sim 7\,^{\circ}C$ at Martian atmospheric pressures.

Martian conditions are extreme, but, at their most hospitable, there are similarities with the most extreme cold regions of the terrestrial biosphere, such as the Dry Valleys or Vestfold Hills of Antarctica. Long regarded as essentially barren of life, recent investigations of Antarctic environments have revealed considerable microbial activity (e.g. Priscu *et al.* 1998). The Archaea and Bacteria that have adapted to these extreme conditions are some of the best candidates for terrestrial analogues of potential extraterrestrial life; understanding their adaptive strategy, and its limitations, will provide deeper insight into fundamental constraints on the range of habitable environments (Cavicchioli 2002; DasSarma 2006).

More than 80% of the Earth's biosphere is permanently at temperatures below $4\,^{\circ}C$. Given the abundance and diversity of Archaea in cold environments (Karner *et al.* 2001; Feller & Gerday 2003), especially in the oceans, our understanding of the genetics and physiology of cold-adapted Archaea is surprisingly scarce (Cavicchioli *et al.* 2000). Some insights have been obtained on the level of protein structure and function (Schleper *et al.* 1997; Thomas & Cavicchioli 2000; Thomas *et al.* 2001; Siddiqui *et al.* 2002), gene regulation (Lim *et al.* 2000; DasSarma 2006), tRNA modification (Noon *et al.* 2004), membrane lipid composition (Nichols *et al.* 2004; Gibson *et al.* 2005), proteomics (Goodchild *et al.* 2004a, b, 2005; Saunders *et al.* 2005) and genomics (Saunders *et al.* 2003) by studying cold-adapted Archaea.

We have been conducting experiments that are designed to test the lower temperature limits for growth of four extremophile species: a model halophile, *Halobacterium* sp. NRC-1; a mesophile, *Halorubrum lacusprofundi*; a cold-adapted halophile from Antarctica; and two halotolerant methanogens, *Methanosarcina acetivorans*, a mesophile, and *Methanococcoides burtonii*, which is cold-adapted. In addition, *Escherichia coli* strain MG1655 was included in this work as a control, representing a well-studied terrestrial bacterium. Both halophiles and methanogens are potentially relevant to life in Martian environments: halophiles could thrive in subsurface brine which remains liquid at temperatures well below $0\,^{\circ}C$ (DasSarma 2006); while the presence of methanogens, which can grow in an anoxic atmosphere, is one of the proposed explanations for the occurrence of significant methane in the present-day Martian atmosphere (Krasnopolsky *et al.* 2004; Mumma *et al.* 2004).

The primary goal of our experiments was to map the full temperature range over which these five species show significant growth, and to compare those limits against the temperature distribution of Martian environments. These species are not capable of growth at temperatures below $-10\,^{\circ}C$, and therefore do not rival bacterial species such as *Colwellia psychrerythraea*, with an extrapolated minimum growth temperature of $-14.5\,^{\circ}C$ (Methe *et al.* 2005). Nonetheless, the behaviour shown by the individual methanogens and halophiles targeted by our investigation may shed light on more general survival strategies adopted by these important archaeal species at low temperatures. The following section outlines the methods adopted in pursuing these experiments and the third section presents our results. We consider the astrobiological implications of the results in the Discussion section and the final section summarizes our conclusions.

Materials and methods

Bacterial strains

E. coli strain MG1655 (=ATCC 47076) was obtained from the collection maintained by RB. The mesophilic haloarchaeon *Halobacterium* sp. strain NRC-1 (=ATCC 700922) was from the culture collection maintained by SD and the cold-adapted haloarchaeon *H. lacusprofundi* strain ACAM 34 (=ATCC 49239) was obtained from ATCC. The mesophilic methanogen *M. acetivorans* strain C2A (=DSM 2834, =ATCC 35395) isolated from a submarine canyon (Sowers *et al.* 1984) has been maintained by KRS in the laboratory and the cold-adapted methanogen *M. burtonii* Ace M (=DSM 6242) isolated from an Antarctic saline lake (Franzmann *et al.* 1992) was obtained from the collection maintained by RC.

Media and growth conditions

The mesophilic bacterium *E. coli* strain MG1655 was grown in Erlenmeyer flasks (1 l) containing 100 ml of Luria Bertani (LB) (10 g of tryptone, 5 g of yeast extract, and 10 g of NaCl per litre) medium (Sambrook *et al.* 1989). Cultures were pre-incubated at the respective growth temperatures in incubator shakers for at least 3 h before the addition of 1 ml of an overnight culture. The mesophilic haloarchaeon *Halobacterium* sp. strain NRC-1 was grown in a complex medium containing peptone ($10\,g\,l^{-1}$) and 10 mM citrate as carbon and energy sources, respectively (CM^{+} medium; DasSarma & Fleischmann 1995). The cold-adapted haloarchaeon *H. lacusprofundi* was grown in an artificial Deep Lake medium, containing succinate and yeast extract as carbon and electron donors, respectively, as described previously (Franzman *et al.* 1988). The halophilic strains were grown in 250 ml Erlenmeyer flasks containing 50 ml of medium and incubated in the light at temperatures indicated either in a refrigerated circulator (Neslab RTE-111, Thermo Electron Cooperation) or in a refrigerated incubator shaker (New Brunswick). The mesophilic methanogen *M. acetivorans* and the cold-adapted methanogen *M. burtonii* were grown in an anaerobic defined minimal marine medium as described by Sowers *et al.* (1993) with 0.05 M trimethylamine. The medium was anaerobically dispensed in 10 ml aliquots into 20 ml Balch anaerobe tubes, which were sealed under N_2-CO_2 (80:20) with butyl rubber stoppers secured with aluminium

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crimp seals. The medium was autoclaved for 20 min and the final pH of the medium was 6.8. Methanogens were incubated in circulating water baths containing ca. 20% glycerol at the temperatures indicated. Medium pre-incubated at the indicated growth temperatures was inoculated with 10% (v/v) of a culture grown at 4 °C.

Analytical methods

Growth was monitored spectrophotometrically by determining the optical density at 600 nm (1 cm path length) for *E. coli*, *Halobacterium* sp. NRC-1 and *H. lacusprofundi*, and at 550 nm (1.8 cm path length) for *M. acetivorans* and *M. burtonii*. Growth of *M. acetivorans* and *M. burtonii* was also monitored by assaying methanogenesis. Methane was sampled from the headspace with a 50 µl gas-tight syringe equipped with a valve and analysed by gas chromatography with an HP5890 (Agilent Technologies, Inc., Palo Alto, CA) configured with a flame ionization detector. The sample was injected onto a 0.32 cm by 182 cm stainless steel column packed with 80:20 mesh 923 grade silica gel (Supelco, Bellefonte, PA) with nitrogen (30 ml min^{-1}) as the carrier gas and run with an isocratic oven temperature of 110 °C. Analytical grade methane was used as a standard. All experiments were stopped when the optical denisty or methane levels of a treatment did not change in three or more consecutive samplings.

Microscopy

Samples for scanning electron microscopy (SEM) imaging were fixed in 2% glutaraldehyde at room temperature for 60 min and then 4 °C overnight. Samples were then filtered onto a 0.2 or 0.4 µm Nuclepore polycarbonate filter and further fixed with a buffered 1% OsO_4 solution for 60 min at room temperature. Following ethanol dehydration, the samples were critical point dried, Au/Pd coated using a Denton vacuum evaporator and imaged on a Hitashi S-4700 FESEM.

Bioinformatic analysis

Sequence data were obtained either from SD's web site (http://zdna2.umbi.umd.edu) or NCBI (National Center for Biotechnology Information). Nucleotide and amino acid sequence analyses were performed using the programs from the GCG Wisconsin software package (Accelrys, San Diego, CA), DNAStar software package (DNASTAR, Madison, WI), Clustal_X (version 1.81) and Simple Modular Architecture Research Tool SMART (http://smart.embl-heidelberg.de).

Experimental results

Growth of methanogens at low temperatures

The effect of low temperatures on growth and methanogenesis was studied with the cold-adapted Archaeon, *M. burtonii*, isolated from an *in situ* temperature of 1.7 °C in Ace Lake, a saline lake in Antarctica (Franzmann *et al.* 1992), and the mesophile *M. acetivorans* isolated from an *in situ* temperature of 12 °C in a submarine trench (Sowers *et al.* 1984). Both growth and methanogenesis were assayed in cultures of *M. burtonii* and *M. acetivorans* grown at and below the reported temperatures for growth in the mineral medium (Sowers *et al.* 1984; Franzmann *et al.* 1992). *M. acetivorans* did not grow or produce methane at 10, 4 or −2 °C after 190 days (Fig. 1(a)) although cells remained viable and growth could be initiated by shifting the incubation temperature of these cultures to 35 °C. In a prior report, no growth was detected for *M. acetivorans* at temperatures of 10 °C or less (Sowers *et al.* 1984). Extended incubation for 300 days at 10, 4 and −2 °C did not result in detectable growth (unpublished data). These results demonstrate that although *M. acetivorans* remained viable at temperatures below 10 °C, it did not continue to replicate at detectable levels.

In contrast, based on an increase in optical density and the production of methane, *M. burtonii* grew at 10, 4 and −2 °C (Fig. 1(b)). Neither growth nor methanogenesis was observed at −5 °C. In prior reports, *M. burtonii* grew at 1.7 °C if the culture was pre-incubated at 20 °C to an optical density (OD_{550}) of 0.3 (Franzmann *et al.* 1992). In this report we show that *M. burtonii* preincubated at 4 °C to an OD_{550} of 0.1 grows near the predicted T_{MIN} of −2.5 °C (Franzmann *et al.* 1992). The addition of the compatible solutes glycine betaine, dimethylsulphoniopropionate or glycerol did not further reduce the T_{MIN} of either methanogenic strain.

M. burtonii grown at 4 °C and below formed cell aggregates that settled to the bottom of the culture tube forming an apparent biofilm on the glass. The aggregates appeared as a network of fibrils extending between cells in SEM micrographs (Fig. 2(a)) and stained slightly red with the polysaccharide-binding dye Congo red (data not shown). When the aggregates were disrupted by vigorously shaking the tube, turbidity was observed as a result of the dispersion of single cells in the culture medium. Aggregates re-formed within a day of continued incubation at the low temperature.

Growth of halophiles at low temperatures

Temperature–growth curves have been generated for *Halobacterium* sp. NRC-1, a sequenced (Ng *et al.* 2000) and genetically tractable mesophilic halophile isolated from Solar salt, and *H. lacusprofundi*, a cold-adapted halophile isolated from Deep Lake, Antarctica (Franzmann *et al.* 1988). The results for *Halobacterium* NRC-1 indicate that this halophile grows well between 15 and 48 °C (Fig. 1(c) and data not shown). At 4 °C, very little growth was observed (less than one cell doubling) and the culture lost its pink colour, which is indicative of the loss of cell viability. No growth was observed below 0 °C.

For *H. lacusprofundi*, the previously reported lowest experimentally determined growth temperature was 4 °C with a theoretical T_{MIN} of 1 °C (Franzmann *et al.* 1988). In the experiments reported here, growth occurred at temperatures down to −1 °C after several weeks of incubation (Fig. 1(d)). The growth lag was significantly shorter after pre-cultivation at 4 °C as compared to 30 °C, suggesting an adaptive

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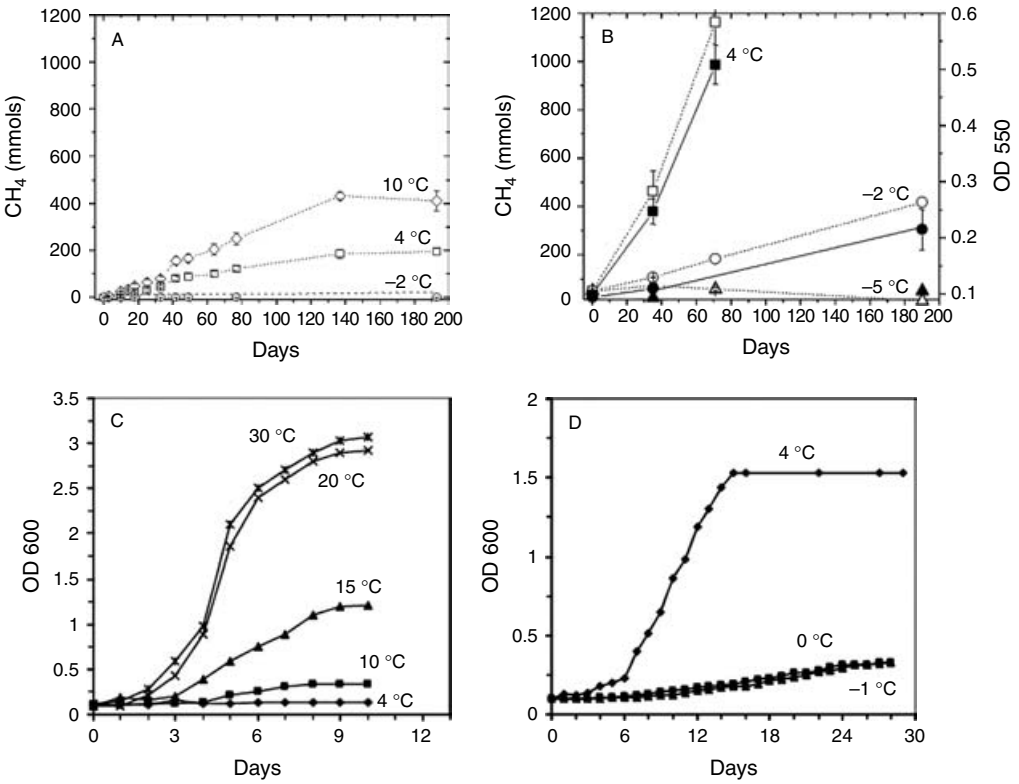

Fig. 1. Growth and methanogenesis at low temperatures of (a) the mesophile *M. acetivorans* and (b) the cold-adapted species *M. burtonii*. Solid lines with solid symbols represent growth based on optical density at 550 nm (OD_{550}) and dashed lines with open symbols represent methanogenesis at 10 °C (◇), 4 °C (□), −2 °C (○) and −5 °C (△). Each datum point is the mean of three replicate cultures. (c) Growth of the mesophile *Halobacterium* sp. NRC-1 (30 °C (*), 20 °C (×), 15 °C (▲), 10 °C (■), 4 °C (◆)) and (d) the cold-adapted *H. lacusprofundi* (4 °C (◆), 0 °C (■) and −1 °C (▲)). Symbols represent growth based on optical density at 600 nm (OD_{600}). Each datum point is the mean of two replicate cultures.

response to cold temperatures. Growth at 4 °C and 30 °C was as described previously (Franzmann *et al.* 1988). No increase in turbidity was seen at temperatures of −2 °C or below.

Strikingly, during growth of *H. lacusprofundi* at low temperatures, extensive cell aggregation and biofilm formation occurred in the cultures (Fig. 2(b)). As described previously (Franzmann *et al.* 1988), cells of *H. lacusprofundi* also form multicellular aggregates during growth at temperatures higher than the optimal growth temperature. Only weak aggregation of cells was observed when cultures were incubated at room temperature for a similar time span. Once formed at a cold temperature, the multicellular structures were stable at room temperatures despite agitation of the culture for 1 day. The temperature-dependent formation of stable cell aggregates suggests that changes associated with the cell envelope occurred during incubation at the lower temperature. Scanning electron micrographs of *H. lacusprofundi* cell aggregates grown at 4 °C revealed a network of fibrils extending from and interconnecting cells (Fig. 2(b)). These fibrils resembled the matrix of exopolysaccharide fibrils present in many microbial biofilms. The presence of extensive polysaccharides in aggregates of *H. lacusprofundi* was shown by staining with the polysaccharide-binding dye Congo red (data not shown).

Escherichia coli *growth at low temperature*

As *E. coli* is a mesophile, the data collected served as a control for comparison to the observations and data collected from the cold-adapted Archaea examined as part of this study. *E. coli* was grown in LB medium at temperatures between 10 and 37 °C, and the growth rate was found to be temperature dependent. Maximal growth was achieved at 37 °C, which included a short lag phase (< 1 h) that resulted in the culture achieving a stationary phase in 3 h. At 20 °C, the lag phase was longer (ca. 2 h) than observed at 37 °C, and the culture required ca. 7 h to reach a stationary phase. At 10 °C, the lowest temperature where growth was measured, the lag period increased to ca. 48 h, and the bacteria required ca. 123 h or 5.1 days to reach a stationary phase of growth. Based on the linear portion of each growth curve (data not shown), the doubling time for *E. coli* grown at 37 °C was 44 min whereas the doubling times of *E. coli* at 20 °C and 10 °C where 100 min (2.3 times slower) and 1856 min (42.2 times slower), respectively. There was no significant growth of *E. coli* at 5 °C over a month-long period.

Bioinformatic analysis

The low-temperature growth capability of *M. burtonii* and *H. lacusprofundi* reflects the genomic composition of these

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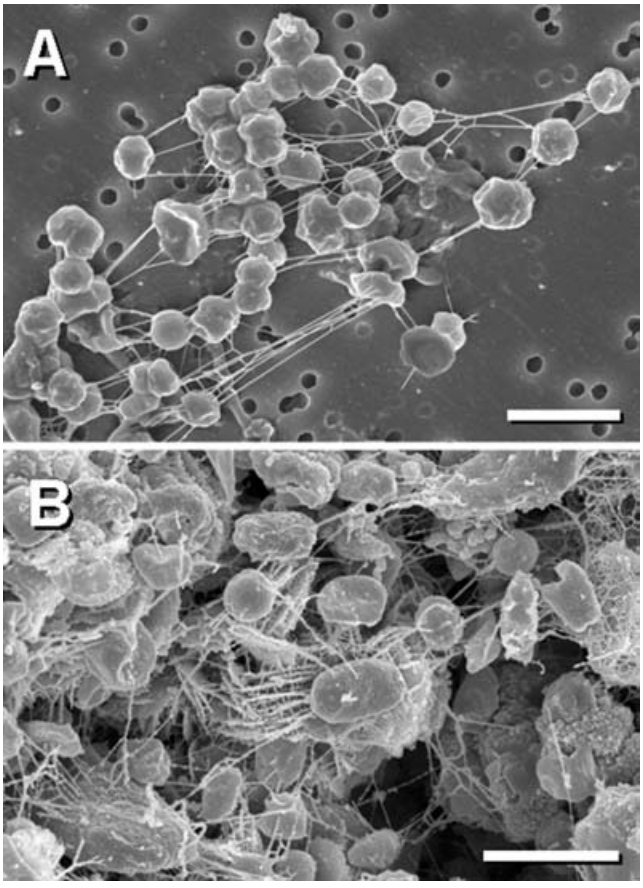

Fig. 2. Scanning electron micrographs of cell aggregates (a) *M. burtonii* grown at −2.5 °C and (b) *H. lacusprofundi* grown at 4 °C. In both cases, the scale bar has length 2 μm.

highly adapted microorganisms. Since partial genome sequences are available for both, we have conducted an inventory of predicted proteins known to be important for coping with low temperatures. Strikingly, the genome of *H. lacusprofundi* is predicted to contain multiple copies of each of the two different cold shock protein (CSP) genes, while the mesophilic *Halobacterium* sp. NRC-1 contains only one copy each (*cspD1* and *cspD2*). In general, CSPs are involved in various cellular processes and they are believed to enable cells to adapt to low temperature (Thieringer *et al*. 1998). They can bind to single-stranded DNA and RNA and have been suggested to function as RNA chaperones facilitating the initiation of translation under optimal and low temperatures. Overproduction of CSPs in an organism can lead to increased survival during periods of freezing (Wouters *et al*. 2001). The presence of multiple copies of CSP genes in *H. lacusprofundi* immediately suggests a possible molecular basis for the cold-adapted nature of this organism.

Likewise, comparative analysis by Saunders *et al*. (2003) of the incomplete genomes of the cold-adapted methanogen *M. burtonii* as well as the psychrophilic species *Methanogeneium frigidum*, both isolated from Ace Lake, also revealed a gene with high sequence identity to the *E. coli* cold shock domain (CSD) protein *cspA* in the genome of *M. frigidum* and two proteins in *M. burtonii* that contain the highly conserved CSD fold characteristic of CSPs. Overall, the genome analyses of these Antarctic halophiles and methanogens indicate that cold-adapted Archaea have evolved specific mechanisms for adapting to low temperatures.

Discussion

Growth conditions of methanogens

Our experiments indicate that, given a sufficient supply of liquid water, conditions on Mars and possibly other planets could support methanogenic Archaea found here on Earth; for example, cold temperatures, minimal medium, low nutrients (oligotrophs), anoxic conditions (Cavicchioli 2002). The methanogenic Archaea generally grow optimally in strictly anaerobic environments with redox potentials below −320 mV (Hungate 1950). Survival and growth under low-redox potential occurs by the catabolism of inorganic or simple organic compounds. These include the reduction of CO_2 with an electron donor such as hydrogen, the dismutation of acetate and subsequent reduction of the methyl group or by the reduction of the methyl group of simple methylated compounds including methylated amines and sulphides (Sowers 2004). Some methanogenic Archaea can also grow by non-methanogenic fermentation of CO (Rother & Metcalf 2004).

Methanogens are generally found in anaerobic, reduced environments where substrates are either provided by other anaerobic microorganisms or from geological sources. Despite their requirement for anoxic conditions, the methanogens as a group exhibit a diverse range of physiological adaptation. These environments can range from geothermal vents at temperatures greater than 100 °C to subzero temperatures in Antarctic lake sediments, freshwater lakes with low solute concentrations to hypersaline salterns or from acidic geothermal calderas to alkaline lakes (Sowers 2004). The methanogenic Archaea often require only inorganic nutrients to support growth. In one terrestrial example, methanogens found in basalt rock during deep subsurface drilling appear to live autotrophically on hydrogen generated from the reaction between ferrous ions and silicates in basalt, providing an analogue for possible subsurface microbial ecosystems on other planets such as Mars and Europa (Chapelle *et al*. 2002). Deep subsurface methanogens growing on hydrogen or simple organic compounds could serve as a primary productivity source for a more complex food web in either diminished sunlight or the complete absence of sunlight that may exist on other planets. Kral *et al*. (2004) showed that several species of mesophilic methanogens could grow in terrestrial volcanic ash with composition, grain size, density and magnetic properties similar to those thought to exist on Mars. The extended temperature range exhibited by *M. burtonii* from −2 °C to 29 °C, which is characteristic of subsurface temperatures on Mars, the ability to grow in a mineral medium with a low-molecular-weight organic substrate and the osmotolerance of this species to 0.5 M NaCl reported previously (Franzmann *et al*. 1992) further support

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the possibility that methanogenic microorganisms could be the source of detected methane.

Growth conditions of extreme halophiles

Extremely halophilic Archaea generally grow optimally in environments with salinities greater than 20% (w/v) (DasSarma & Arora 2002; DasSarma 2006). Survival and growth under these hypersaline conditions are accomplished by maintaining an isoosmotic balance of the cytosol with the surrounding medium. Haloarchaea accumulate KCl equal to external NaCl and other salt concentrations. Recently, haloarchaea have been discovered in deep hypersaline anoxic basins in the Mediterranean Sea, which are some of the most extreme terrestrial saline environments known as they are almost saturated with $MgCl_2$ (van der Wielen *et al.* 2005). Haloarchaea are also well adapted to high concentrations of $MgSO_4$, which is widely distributed within saline sediments on Mars (Vaniman *et al.* 2004).

Haloarchaea can derive metabolic energy for growth through a number of pathways: chemoorganoheterotrophically, using a plethora of organic electron donors; via aerobic as well as anaerobic respiration, using inorganic nitrogen oxides and organic electron acceptors (DMSO, TMAO); or through the fermentation of simple organic compounds (Oren & Trüper 1990; DasSarma & Arora 2002; Müller & DasSarma 2005). In addition, inorganic sulphur compounds have been suggested to serve as potential electron acceptors (Tindall & Trüper 1986), but little information is available on the nature of the process. Inorganic sulphur compounds have been detected on the surface of Mars (Fairen *et al.* 2004).

Haloarchaea grow phototrophically, employing the proton pump bacteriorhodopsin. This capability has recently been discovered among a wide variety of Archaea, bacteria, and Eukaryotes and may represent an ancient form of metabolism, which may possibly have co-evolved with chlorophyll-based photosynthesis (Wickramasinghe 1976; Spudich *et al.* 2000; Bielawski *et al.* 2004; DasSarma 2006). The ability of extremely halophilic Archaea to thrive under anoxic conditions renders this group of organisms suitable as models for a variety of extraterrestrial habitat scenarios. Their potential for primary productivity and their metabolic versatility would present an important part in nutrient cycling.

The present studies with *H. lacusprofundi* have expanded the temperature range supporting growth of that organism. *H. lacusprofundi* was originally isolated from the hypersaline Deep Lake, Antarctica (Franzmann *et al.* 1988), where the lake temperature is below 0 °C for more than half the year (Barker 1981). Deep Lake, located in the Vestfold Hills, is ice-free through out the year due to a freezing point depression from hypersalinity (28% salinity). The surface temperatures have been found to be as low as −20 °C in the winter and up to 10 °C in the summer. Nutrient concentrations are similar to seawater, but there is no dissolved sulphate, probably due to precipitation as mirabilite ($Na_2SO_4 \cdot 10H_2O$). Preliminary bioinformatic analysis suggests that *H. lacusprofundi* is adapted to growth at cold temperatures at least partially due to the increased presence of cold shock genes.

Formation of multicellular structures

It has been suggested that cell aggregation might be a common high temperature-dependent stress response among the Archaea (Hartzell *et al.* 1999). Some *Methanosarcina* spp., form multicellular aggregates (packets of cells) with a resistant methanochondroitin outer layer that is structurally similar to connective tissue of vertebrate animals, when they are grown at low salt concentrations (Sowers *et al.* 1984, 1993). In contrast, when adapted to growth on high salt they only produce an S-layer (surface layer) and do not form packets. Interestingly, we also observed extensive cell aggregation of both *H. lacusprofundi* and *M. burtonii* when cultures were incubated in the cold (Fig. 2). This is of particular interest since the formation of a multicellular complex, comprised of archaeal, bacterial or eukaryotic cells, constitutes one of the most fundamental aspects of developmental biology. Cell aggregation may facilitate the exchange of nutrients, membrane components and genetic material, thus enabling the organisms to cope and grow in an otherwise stressful environment. The euryarchaeon SM1 lives in close association with a *Thiotrix* sp. of bacteria in the cold (~10 °C) sulphurous marsh water (Rudolph *et al.* 2001; Moissl *et al.* 2002). A highly enriched culture of the euryarchaeon SM1 remained viable at temperatures ranging from −2 °C to 20 °C (Moissl *et al.* 2003). The cells are encased in a polymeric matrix of undefined chemical composition (Rudolph *et al.* 2001), which appears to be synthesized by euryarchaeon SM1 as a pili-like fibre that emanates radially from the cell mediating cell–cell and possibly cell–surface interactions (Moissl *et al.* 2003). Sea-ice microorganisms, including *Colwellia psychrerythraea*, are also reported to produce extracellular polymeric substances (EPS) as buffering and cryoprotective agents at temperatures as low as −20 °C (Krembs *et al.* 2002; Methe *et al.* 2005). Collectively, these data indicate that synthesis of extracellular materials has evolved as a mechanism of cold adaptation in phylogenetically diverse microorganisms.

Relevance to extrasolar planetary environments

At present, the lowest-mass planetary companions detected in radial velocity surveys of normal stars have masses close to that of Uranus and Neptune (~7% that of Jupiter), while most have masses comparable to or exceeding that of Jupiter. It is likely that these are gas giants, and therefore inhospitable to microbial life. However, all of the gas giants in the Solar System have relatively massive rocky moons, and such objects might offer suitable platforms for life in the known extrasolar planetary systems. How do the surface temperatures of those hypothetical companions match the growth limits of the species discussed in this paper?

All of the planetary host stars in the Solar Neighbourhood have milliarcsecond-accuracy parallax measurements, and therefore well-determined distances and luminosities (see, e.g., Schneider 2006). Consequently, we can estimate the

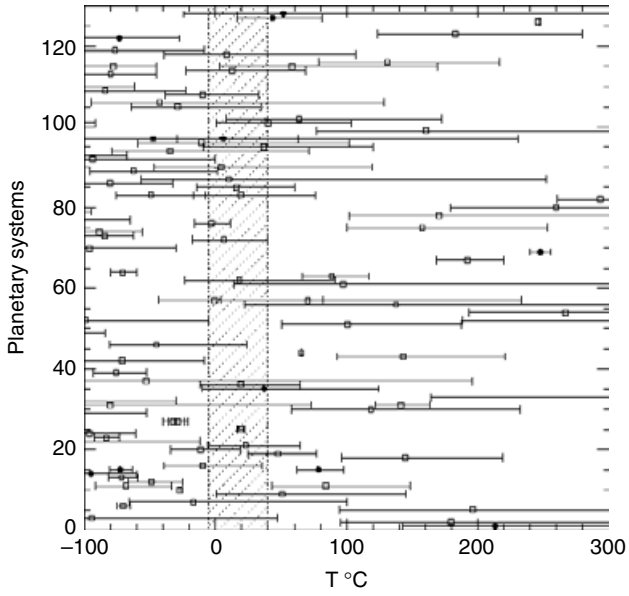

Fig. 3. Predicted temperatures of terrestrial analogues as satellite companions of known extrasolar planets: the ordinate is a sequential numbering of the known systems; open squares mark systems where the primary star is on the main sequence; solid points are planetary companions of giants or subgiants. The error bars show the temperature range expected owing to orbital eccentricity. The shaded region marks the approximate growth limits of the psychrotrophs included in the present set of experiments.

likely conditions on hypothetical satellite moons in those systems, since the ambient temperature depends on the luminosity of the parent star, the distance of the extrasolar planet (or moon) from the central star and the atmospheric properties of the planet or moon. The last is clearly a matter of conjecture, but Fig. 3 shows the temperature range experienced by satellites that are exact analogues of the present-day Earth; that is, we scale current terrestrial conditions based on the incident flux from the central star in those systems. These temperature estimates only take into account insolation effects; tidal heating, as in the Jupiter/Europa system, could lead to habitable temperatures in systems at larger distances from the parent star. Fig. 3 also shows the growth limits of the cold-adapted halophile and methanogen species studied in this set of experiments. Several of the host stars have evolved onto either the subgiant or giant branch, and planets in those systems are undergoing a rather severe form of global warming.

There are two particularly significant features of the temperature distribution shown in Fig. 3. First, relatively few of the hypothetical Earth analogues (16 of 150 planets) have average temperatures that fall within the growth limits of the cold-adapted Archaea discussed here; the majority would have average temperatures well below $-5\,^{\circ}C$. This preponderance of lower temperature systems highlights the importance of extending the current set of experiments to microbes with enhanced growth characteristics in colder temperatures. Second, most systems show substantial variations in temperature (indicated by the error bars in Fig. 3); this reflects the significant orbital eccentricities. Consequently, most (hypothetical) moons with formal average temperatures that fall within the limits spanned by terrestrial life also experience significant temperature excursions, and lie beyond those limits for significant portions of their orbital cycle. If such systems are representative, this suggests that further experiments aimed at determining how well microbial life survives variable temperature environments would be of great interest.

Summary and conclusions

We have presented results from a series of experiments designed to investigate the low-temperature growth limits of selected methanogenic and halophilic archaeal species. We find that the halophile, *H. lacusprofundi*, is active at $-1\,^{\circ}C$, while the methanogen, *M. burtonii*, shows significant growth at $-2\,^{\circ}C$; in both cases, these measurements extend the low-temperature limits reported for these species by several degrees. Both *H. lacusprofundi* and *M. burtonii* show a distinct change of morphology at low temperatures, clumping in aggregates embedded in a network of fibrils. The fibril network is reminiscent of the EPS that some bacteria use to resist and survive extreme conditions, introduced by processes such as low temperature, desiccation or salinity fluctuations. This is the first detection of this phenomenon in Antarctic species of Archaea at cold temperatures. Finally, we have compared the temperature limits for the cold-adapted species, derived from these experiments, against the surface temperatures predicted for terrestrial analogue satellites of the known extrasolar planets. A sizeable proportion of known systems include planets that intersect the Habitable Zone. In most cases, those planets follow orbits with significant eccentricity, leading to substantial seasonal temperature excursions. However, a handful of the known gas giant exoplanets could potentially harbour habitable terrestrial moons.

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