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Survival of non-psychrophilic methanogens exposed to martian diurnal and 48-h temperature cycles

R.L. Mickol [a,d], Y.A. Takagi [b], T.A. Kral [a,c,*]

[a] Arkansas Center for Space and Planetary Sciences, University of Arkansas, Fayetteville, AR, United States
[b] Department of Biology, Oberlin College, Oberlin, OH, United States
[c] Department of Biological Sciences, University of Arkansas, Fayetteville, AR, United States
[d] American Society for Engineering Education, Washington, DC, United States

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ABSTRACT

Polygonal ground and other geomorphological features reminiscent of recent freeze/thaw cycling are evident on Mars, despite the widespread belief that the planet is currently inhospitably cold and dry. On Earth, permafrost microbial communities are subjected to wide ranges in temperature and are often active at subfreezing temperatures. The existence of active microbial communities within permafrost on Earth suggests that permafrost on Mars may constitute a habitable environment.

Terrestrial microbial permafrost communities typically contain methane-producing Archaea, which is cause for concern as global temperatures rise, resulting in permafrost thaw and the release of the potent greenhouse gas. Similarly, on Mars, the overlap between patterned ground and detections of localized methane plumes suggest that the compound may have been released from thawing permafrost.

Analyses of permafrost ice cores and soil samples on Earth note that (1) archaeal communities often contain both mesophiles and psychrophiles at different depths and (2) active methane is being produced at subfreezing temperatures over geological timescales. Thus, the purpose of the experiments described here was to determine the effect of extreme temperature changes (reminiscent of the martian diurnal temperature cycle) on the growth and survival of four non-psychrophilic methanogens previously used as models for potential life on Mars. The results indicate that non-psychrophilic methanogens are capable of survival during extreme diurnal and 48-h temperature changes, similar to those on Mars.

1. Introduction

Mars experiences wide temperature variations over one sol, often ranging from temperatures just above freezing to −100 °C and lower (Kieffer et al., 1977). Any microorganisms that could potentially inhabit Mars would at least need to be able to survive these temperatures, but also make use of any available liquid water or temporary increases in temperature in order to metabolize. Due to the very thin atmosphere and lack of other insulating factors, temperatures vary widely on Mars based on location and season. Temperatures from the primary Viking mission ranged between −143 °C and 17 °C (Kieffer et al., 1977), while measurements from the Thermal Emission Spectrometer (TES) depicted nighttime temperatures ranging between −123 °C and −53 °C (Christensen et al., 2001).

On Earth, methanogenic Archaea within permafrost communities are considered fairly active at subfreezing temperatures [1–2 nmol CH_4/kg/day at −16.5 °C (Rivkina et al., 2004, 2007); 0.04–0.68 nmol CH_4/h/g at −3 °C to −6 °C (Wagner et al., 2007)] and the same could potentially be true for regions on Mars (Gilichinsky et al., 2007; Steven et al., 2009; Wagner et al., 2002). Terrestrial permafrost communities are also subjected to wide variations in temperature over a particular season and may provide insight into the habitability of martian permafrost. On Earth, permafrost is defined by three temperature-dependent layers: the uppermost layer is considered the active layer, experiencing the widest range in temperatures (−50 °C–30 °C) and ranging in thickness between 0.2 m and 2 m. Perennially-frozen permafrost sediments (10–20 m thick) constitute the middle layers and are subject to smaller temperature variations (−15–0 °C above the zero annual amplitude [constant temperature]). Lastly, deeper permafrost sediments are characterized by more stable temperatures, ranging between −10 and −5 °C (Wagner,

* Corresponding author. University of Arkansas, Department of Biological Sciences, SCEN 601, Fayetteville, AR 72701, United States.
E-mail address: tkral@uark.edu (T.A. Kral).

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2008; Wagner et al., 2002). Microbial communities within the active layer are thus subject to yearly freezing and thawing, correlating with seasonal temperatures. Although these temperatures are considerably higher than those currently measured on Mars, survival and growth tactics utilized by microorganisms within permafrost on Earth may provide a useful comparison to the possibility of life on Mars, specifically if the planet may have been slightly warmer in the past (Soare et al., 2008).

Although certain psychrophilic organisms are capable of active metabolism below freezing temperatures where water may still remain liquid, most organisms require temperatures above 0 °C for active growth, and currently, there are no known organisms that can actively grow below −20 °C (Clarke, 2014). Additionally, growth may be relatively slow at lower temperatures (Franzmann et al., 1997; Rivkina et al., 2000) due to reduced molecular kinetic energy and the subsequent lower rate of metabolic reactions (Clarke, 2014). The freezing temperatures of Mars may not be conducive to continued active metabolism, but exposure to these temperatures is not necessarily lethal to the organism. Many organisms have freeze tolerances that enable them to survive freezing temperatures down to −196 °C (Clarke, 2014). Active metabolism is then resumed once warmer temperatures are achieved.

As on Earth, temperatures may be more stable under the martian surface due to insulation from the overlying layers (Henry, 2007). However, stability does not necessitate warmer temperatures. The globally-averaged annual mean soil temperature on Mars is −69 °C, varying between −39 °C at the equator and −114 °C at the poles (Mellon et al., 2004, 2008). Although surface temperatures do rise above 0 °C at the equator, it is believed that near-subsurface (within 6 cm) soil temperatures never rise above freezing (Mellon et al., 2008). However, models utilizing different thermal conductivities and soil densities suggest that temperatures above 0 °C may be possible 3–7 km beneath the martian surface (Mellon and Phillips, 2001). Additionally, on Earth, consistent snowpacks can insulate soil communities and result in higher microbial activities than soils that are not well-insulated, and thus, subject to colder temperatures (Henry, 2007). However, on Mars, only the polar caps would constitute semi-permanent insulated habitats.

Despite the consistently cold temperatures on the planet, Page (2007) notes the existence of various geomorphological landforms that are reminiscent of relatively recent freeze/thaw cycles on Mars. Interestingly, the author suggests that thawing permafrost may result in the loss of volatiles from the soil and thus could form a source for the localized methane plumes detected over the Cerberus plains on Mars – a region that also features polygonal ground (Page, 2007). Additional analyses focusing on martian surface features have also concluded that certain geomorphologies appear to indicate relatively recent freeze/thaw episodes within the last 4 My (Soare et al., 2008; Gallagher et al., 2011; Johnsson et al., 2012). Further, Ulrich et al. (2012) have also noted periglacial landforms on Mars and suggest that martian permafrost may constitute one of the more habitable environments on Mars.

Methanogens are microorganisms in the domain Archaea, most of which utilize hydrogen (H_2) and carbon dioxide (CO_2) to produce methane (CH_4) and are often prominent members of microbial permafrost communities on Earth (Blake et al., 2015; Gilichinsky et al., 2007; Kobabe et al., 2004; Koch et al., 2009; Liebner et al., 2015; Rivkina et al., 2007; Shcherbakova et al., 2016; Wagner et al., 2005, 2007). The discovery of methane in the martian atmosphere (Fonti and Marzo, 2010; Formisano et al., 2004; Geminale et al., 2008, 2011; Krasnopolsky et al., 1997, 2004; Maguire, 1977; Mumma et al., 2009; Webster et al., 2015) reinforces the study of methanogens as candidates for life on Mars. A few studies have assessed the growth and survivability of methanogens under low temperature conditions, focusing on methanogens isolated from permafrost habitats in comparison with both psychrotolerant and non-psychrophilic methanogens from non-permafrost environments (Morozova et al., 2007; Morozova and Wagner, 2007; Schirmack et al., 2014). Morozova et al. (2007) compared survival within pure cultures under martian conditions for three methanogen strains isolated from permafrost and three reference organisms: *Methanosarcina barkeri*, *Methanogenium frigidium* (isolated from Ace Lake, Antarctica) and *Methanobacterium* spec. MC-20. This study consisted of 22 days of diurnal freeze/thaw cycles between −75 °C and 20 °C. Morozova et al. (2007) discovered that the three permafrost strains had the highest survival (60.6%–90.4%, cell counts), whereas the survival rate for the reference organisms was exceptionally low (5.8% survival for *M. frigidum*, 1.1% survival for *Methanobacterium* spec. MC-20, 0.3% survival for *M. barkeri*). Additionally, methane production following exposure was significantly decreased for the three reference strains, whereas the permafrost strains had similar methane production before and after exposure to the freeze/thaw cycles (Morozova et al., 2007). In a separate study, Schirmack et al. (2014) demonstrated methane production by *Methanosarcina soligelidi* (Wagner et al., 2013), a permafrost methanogen also used in the Morozova et al. (2007) study, at temperatures down to −5 °C and at a pressure of 50 kPa (0.5 bar), which the authors suggest may be achievable in the martian subsurface.

Analysis of methane within permafrost ice cores has also led to the suggestion that methane is being produced at subfreezing temperatures [-9 °C, −11 °C] (Tung et al., 2005, 2006) and [-3 °C, −6 °C] (Wagner et al., 2007). Tung et al. (2005) analyzed gas concentrations within glacial ice cores and calculated average metabolic rates associated with excess methane using cell counts within the ice. The authors suggest that this metabolic energy is typically expended to repair damaged DNA and amino acids (Tung et al., 2005). These findings correspond to the idea of "survival metabolism", proposed by Morita (1997) and illustrated by Price and Sowers (2004). Morita (1997) suggested that, for starving, non-growing microbes particularly, two types of metabolism could be occurring: survival metabolism and maintenance metabolism. Maintenance metabolism refers to any energy expended for "osmotic regulation, maintenance of intracellular pH, futile cycles, turnover of macromolecules, motility, energy dissipation by proton leak and ATP hydrolysis" but specifically precludes biomass production (Price and Sowers, 2004). On the other hand, survival metabolism refers to energy used solely to repair macromolecular damage (Price and Sowers, 2004). In both cases, the microorganisms are technically "alive" and "actively metabolizing" but neither growing nor increasing in biomass. Price and Sowers (2004) indicate that microbes undergoing maintenance metabolism typically have more access to nutrients and are free to move, whereas survival metabolism is evident as a survival tactic for microbes in deep glacial ice, subsurface sediments, and ocean sediments. From their analysis, the authors also suggest that organisms may be capable of survival metabolism at temperatures down to −40 °C (Price and Sowers, 2004). Thus, for methanogens in particular, cells in frozen sediment or permafrost undergoing freeze/thaw cycles may be actively undergoing survival metabolism, but not increasing in cell density. As such, freeze/thaw cycles within subfreezing environments on Mars may prove relatively habitable in terms of survival of extant organisms.

The experiments described here exposed four methanogen species (*Methanothermobacter wolfeii*, *Methanobacterium formicicum*, *Methanosarcina barkeri*, *Methanococcus maripaludis*) to temperature changes between −80 °C and 22 °C using both 24-h and 48-h cycles. These experiments attempted to expose non-psychrophilic species of methanogens to temperature cycles relevant to Mars.

2. Materials and methods

2.1. Microbial procedures

Methanogens were originally obtained from the Oregon Collection of Methanogens (OCM), Portland State University, Oregon. Each methanogen was initially cultured in its respective anaerobic growth medium and kept at a temperature within the organisms' ideal growth range: *Methanococcus maripaludis* (OCM 151), MSH medium (Ni and Boone, 1991), 22 °C; *Methanosarcina barkeri* (OCM 38), MS medium (Boone et al., 1989), 37 °C; *Methanobacterium formicicum* (OCM 55), MSF medium (MS medium supplemented with formate), 37 °C;