

METHANOGENS AS MODELS FOR LIFE ON MARS. R. L. Mickol¹, W. H. Waddell², and T. A. Kral^{1,3},

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Introduction: The discovery of methane in the martian atmosphere [1-4] has fueled the study of methanogens as ideal candidates for life on Mars. Methanogens are chemoautotrophs from the domain Archaea. These microorganisms utilize hydrogen as an energy source and carbon dioxide as a carbon source to produce methane. Methanogens can be considered ideal candidates for life on Mars because they are anaerobic, they do not require organic nutrients and are non-photosynthetic, indicating they could exist in subsurface environments.

Our lab has studied methanogens as models for life on Mars for the past 20 years [5-12]. Previous experiments have demonstrated the ability of methanogens to metabolize at low pressure on JSC Mars-1 [10] and in the presence of perchlorates [11], as well as survival following desiccation at both Earth and Mars surface pressures (1 bar and 6 mbar, respectively) [10], in the presence of two martian soil simulants (JSC Mars-1 and montmorillonite) [12].

Here we present further evidence for methanogen growth and survival under martian conditions, including survival at low temperature [13, 14], survival at low pressure [15], growth in the presence of Mojave Mars Simulant (MMS) [16, 17], a martian soil simulant, and growth in the presence of montmorillonite, a clay found on Mars [18].

Methods: The four species of methanogen tested include *Methanothermobacter wolfeii*, *Methanosarcina barkeri*, *Methanobacterium formicicum* and *Methanococcus maripaludis*.

Low temperature (freeze/thaw): Two methanogen species were used in these experiments: *M. wolfeii* and *M. formicicum*. Methanogens were grown in their respective anaerobic growth media and inoculated into fresh media (5-10 mL) containing 0 to 10 g sand (silica) or 0 to 5 g gravel, comprising four separate sets. The tubes were pressurized with 200 kPa H₂ and subjected to freeze/thaw cycles between the organisms' ideal growth temperatures (37°C, *M. formicicum*; 55°C, *M. wolfeii*) and -80°C. Transfers to new media were performed during the experiment at least one for each set. Growth was monitored over time by methane production measured via gas chromatograph.

Low pressure: Low pressure experiments were conducted in the Pegasus Planetary Simulation Chamber at the Arkansas Center for Space and Planetary

Sciences, University of Arkansas, Fayetteville, Arkansas. All four methanogen species were tested in these experiments. Methanogens were grown in their respective anaerobic growth media and placed into the chamber with a palladium catalyst box to remove residual oxygen. The chamber was evacuated to a predetermined pressure and filled with 80:20 H₂:CO₂ gas. This procedure was repeated three times to ensure removal of the atmosphere. The chamber was then maintained at the desired pressure (133-143 mbar, 67-72 mbar, 33-38 mbar, 6-10 mbar, 7-20 mbar) for the duration of the experiments. The tubes were punctured with a specialized device after one or two days to equilibrate the tubes with the atmosphere within the chamber. The chamber was filled to atmospheric pressure with CO₂ gas before the tubes were un-punctured and removed. Following removal, 0.5 mL was transferred from the original tubes to tubes containing fresh media. The organisms were incubated at their respective ideal growth temperatures (37°C, *M. barkeri*; 24°C, *M. maripaludis*). Growth was monitored by methane production and optical density.

In one experiment, JSC Mars-1 was used as a diffusion barrier to slow evaporation. The methods are the same as above except that a sterile cotton ball was placed above the liquid medium, with 5 g sterile JSC Mars-1 resting on top. These tubes were then stoppered and crimped before being placed in the chamber.

Mojave Mars Simulant: All four methanogen species were tested in these experiments. Methanogens were grown in their respective anaerobic growth media and inoculated into tubes containing fresh media and 10 g sterile Mojave Mars Simulant. Growth was monitored over 140 days by methane production measured via gas chromatograph.

Montmorillonite: Only *M. maripaludis* was tested in this experiment as it was the only species that showed limited growth in the presence of montmorillonite in preliminary experiments. Methanogens were subjected to 8 conditions consisting of altered MSH medium [19] (including a control without any montmorillonite) in order to determine the minimum conditions for growth in the presence of the clay. All tubes contained 0.5 g montmorillonite (except the control) and 10 mL of either MSH medium, buffer, salt solution, or an altered MSH medium. Growth was monitored over 15 days by methane production.

Results:

Low temperature (freeze/thaw): At least one replicate from each of the four original sets, as well as the transfer sets, survived (contained actively metabolizing methanogens) the duration of the experiment, except for the 5 g sand + 5 g gravel transfer set. Methane production typically decreased over time with exposure to cold temperatures (4°C, -15°C, -80°C), however cultures rebounded after being placed at either room temperature or incubation temperature (37°C for *M. formicicum*, 55°C for *M. wolfeii*). Occasionally, methane abundances would increase in one or more tubes at 4°C or -15°C (see Discussion).

Low pressure: Living cells of all four methanogen species survived exposure to low pressure between 143 mbar and 6 mbar. The limiting factor in these experiments is evaporation of the liquid medium. In the experiment containing JSC Mars-1 as a diffusion barrier, the regolith simulant prolonged the experiment at 6 mbar an extra two days by slowing the rate of evaporation.

Mojave Mars Simulant: Growth was similar for *M. wolfeii* and *M. formicicum* in both the presence and absence of MMS, but with slightly higher methane production in tubes without MMS. *M. maripaludis* was the only organism where the presence of MMS significantly hindered methane production by the organism (compared to growth in normal media). In contrast to the other three organisms, *M. barkeri* was the only methanogen that produced greater amounts of methane in the presence of MMS than in the absence of MMS.

Montmorillonite. Two tested conditions containing montmorillonite showed greater methane production than the control (MSH medium without montmorillonite). The conditions of MSH-Solution D and MSH-Solution B produced the most methane (20% headspace and 24% headspace, respectively), with the control tubes only producing about 10% methane. However, for MSH-Solution D and MSH-Solution B, one of the three replicates produced much less methane than the others, contributing to large error bars. Three conditions all produced about 5% methane (MSH-Solution A, Salt Solution, MSH). Two conditions resulted in no growth, MSH-Salts and NaOH buffer.

Discussion: The survival and growth of methanogens subjected to various simulated martian conditions increases the validity of methanogens as models for life on Mars. However, in reality, microorganisms would be subjected to these conditions concurrently, with synergistic effects likely over-stressing the organisms [20]. Schuerger et al. (2012) identified 14 biocidal factors that any extant life on Mars would need to endure [20, 21]. In addition, the experiments described here all assume liquid water is available, at least within

the near subsurface. The UV radiation on Mars has been shown to be sterilizing, although just 1 mm of soil would serve to fully attenuate this biocidal factor [21]. Thus, another assumption the above experiments make is sufficient protection from UV radiation. This is not unlikely due to the non-photosynthetic nature of methanogens, indicating that they could survive in subsurface environments.

In the low temperature experiments, methane abundances increased at low temperatures believed to be inhibitory to metabolism (-15°C) in one or more replicates. The solubility of methane in water is too low to account for these increases. In addition, the number of CH₄ molecules adsorbed onto regolith decreases exponentially with increasing temperature, approaching zero molecules adsorbed near 145 K [22], therefore this is an unlikely scenario. However, these results require future study regarding active metabolism and methane solubility/adsorption.

Conclusions: The survivability and growth of four methanogen strains under the simulated martian conditions shown here warrants further investigation into methanogens as candidates for life on Mars. Although the microorganisms were tested under these conditions separately, future experiments could focus on the synergistic effects of two or more of these conditions.

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