SIMULATING MARTIAN CONDITIONS: METHANOGEN SURVIVABILITY DURING FREEZE-THAW CYCLES. S. Djordjevic[1,2], R. L. Mickol[1], T. A. Kral[3], [1]Arkansas Center for Space and Planetary Sciences, University of Arkansas, Fayetteville, Arkansas, rmickol@uark.edu [2]University of Illinois at Urbana-Champaign, Champaign, Illinois, djordje2@illinois.edu, [3]Department of Biological Sciences, University of Arkansas, Fayetteville, Arkansas, tkral@uark.edu

Introduction: Methanogens are obligate anaerobes that use molecular hydrogen as an energy source and carbon dioxide as a carbon source to produce methane. They are classified as Archaea and are found in many extreme environments, including hydrothermal vents, volcanoes, and also the human microflora. The current Martian atmosphere is low in pressure, very dry (hyper-arid), and high in radiation, and thus the surface is not suitable for life. However, the subsurface contains permafrost, liquid water [1], and trace amounts of methane [2, 3]. Thus, it is proposed that these Archaea are able to persist in Martian conditions.

According to data obtained from NASA's Mars Science Laboratory between August 2012 and the present time, the maximum and minimum temperatures on Mars have ranged from +20°C to -100°C (Fig. 1) [4]. These conditions might be suitable for methanogenic growth. The goals of this experiment are to use freeze-thaw cycles and measure methanogen growth using gas chromatography in order to further understand temperature constraints on growth.

Methods: Four types of methanogen growth media were prepared according to Kendrick and Kral [5]. The strains used were *Methanothermobacter wolfeii, Methanosarcina barkeri, Methanobacterium formicicum*, and *Methanococcus maripaludis.* The media were autoclaved for sterilization, inoculated with 0.5 mL of each respective methanogen, and grown in their respective media and optimal temperature at ambient pressure: *M. wolfeii* at 55°C, *M. barkeri*/*M. formicicum* at 37°C, and *M. maripaludis* at room temperature (22°C). Growth was monitored in the form of methane production throughout the duration of the experiments via gas chromatography.

Experiment 1. Following seven days at their respective incubation temperatures, all four of the methanogen inoculums were exposed to 4°C for seven days and incubated for six days, respectively (Fig. 2).

Experiment 2. Each inoculum was placed in either five grams of sand or ten grams of sand. Following fifteen days at their respective incubation temperatures, all four of the methanogen inoculums were exposed to 4°C for eight days, -20°C for eleven days, -80°C for eight days, and then incubated for seven days, respectively (Fig. 3).

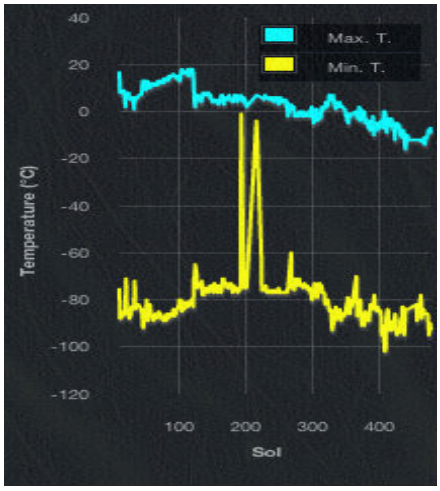

Figure 1. Ground temperature data from the Rover Environmental Monitoring System from early-August 2012 to the present time at Mars' Gale Crater [4].

Results: Only methane concentrations above 1% were considered.

Experiment 1. Following seven days of incubation at their respective temperatures, *M. wolfeii*, *M. maripaludis*, and *M. formicicum* showed appreciable growth after exposure to 4°C and incubation. *M. barkeri* did not show appreciable growth during the experiment and these data were not included (Fig. 2).

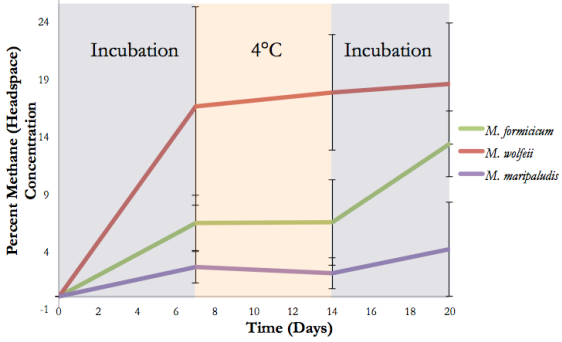

Figure 2. Percent methane (headspace) concentrations for each of three methanogen strains (*M. wolfeii*, *M. maripaludis*, *M. formicicum*) following a seven day incubation period, a 4°C freeze-thaw cycle, and subsequent incubation period.

Experiment 2. Following fifteen days of incubation at their respective temperatures, *M. formicicum* showed appreciable growth only with ten grams of sand at -20°C. *M. wolfeii* showed increased methane production at -20°C and the ten gram

inoculum produced marginally higher levels of methane. *M. maripaludis* did not show appreciable growth and these data were not included (Fig. 3).

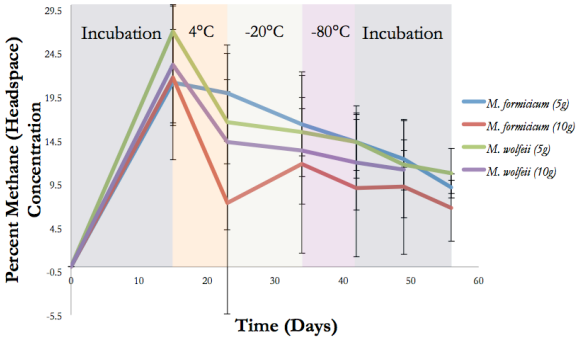

Figure 3. Percent methane (headspace) concentrations for each of two methanogen strains (*M. wolfeii* and *M. formicicum*) in five and ten gram inoculums following a seven day incubation period and various freeze-thaw cycles.

Discussion: *M. wolfeii* showed the largest increase in growth during the incubation period for Experiment 1 and Experiment 2. In Experiment 2, *M. wolfeii* did not show growth at any temperature (besides incubation). The decrease in growth at 4°C was not an expected result because this species showed growth at 4°C in Experiment 1. This result might be due to errors in the measurements of methane concentrations. Thus, the -20°C to -80°C temperature range might be a temperature constraint for this species.

M. formicicum showed an increase in growth during the incubation period for Experiment 1 but only an increase in growth during the -20°C in Experiment 2. Thus, *M. formicicum* might be able to survive lower temperatures more easily than *M. wolfeii*. In Experiment 2, *M. formicicum* grew slightly at -20°C but did not grow at -80°C. Thus, the -20°C to -80°C temperature range might be a temperature constraint for this species.

M. maripaludis showed only marginal changes in growth during Experiment 1. This result might be due to improper lab technique.

M. barkeri did not show appreciable growth during either experiment. This result might be due to improper lab technique and/or low survivability.

It is important to note that decreases in methane concentration were expected as samples were removed from the test tubes for testing. Increases in methane concentration were not expected at low temperature (indicating active growth) because methane is poorly soluble in water at low temperatures [5]. These two factors contribute to decreases in methane concentrations over time.

Conclusion: Four different species of methanogen were used to analyze the effect of low temperature freeze-thaw cycles on the growth of each of the respective species. These experiments provide preliminary data for the growth of *M. wolfeii*, *M. formicicum*, *M. barkeri*, and *M. maripaludis* in low-temperature freeze-thaw cycles. This study has shown that some, but not all, of the strains of methanogen used in this study can withstand prolonged low-temperature conditions, for at least one week at a time. Further studies will continue to analyze the temperature constraints for these Archaea in order to understand implications for life in Martian conditions. Long-term freeze/thaw cycle experiments using *M. wolfeii* and *M. formicicum* were also conducted (R.L. Mickol and T.A. Kral, LPSC XLV, this conference).

References: [1] S.W. Squyres et al. (2006) *Science* 306, 1709–1714. [2] V. Formisano et al. (2004) *Science* 306, 1758–1761. [3] V.A. Krasnopolsky et al. (2004) *Icarus* 172, 537–547. [5] Rover Environmental Monitoring Station (REMS) aboard the Mars Science Laboratory (2013), courtesy of NASA/JPL-Caltech/CSIC-INTA. [4] Kendrick, M.G. and Kral, T.A. (2006) *Astrobiology*, 6, 546–551. [5] Duan, Z. and Mao, S. (2006) *Geochimica et Cosmochimica Acta* 70, 3369-3386.

Acknowledgements: Special thanks to Dr. Julia Kennefick and NSF Grant #1157002.