Freeze Tolerances of Four Non-psychrophilic Methanogens. J. Chuang[1], R. L. Mickol[2] and T. A. Kral[1,2], [1]Dept. of Biological Sciences, University of Arkansas, SCEN 601, Fayetteville, AR, 72701, [jrchuang@uark.edu]; [2]Arkansas Center for Space and Planetary Sciences, University of Arkansas, 332 N. Arkansas Ave, Fayetteville, AR, 72701.

Introduction: The harsh conditions of Mars, such as the very thin atmosphere and frigid temperatures, are two such potentially biocidal factors that any extant life on the planet would need to survive. Recent discoveries have revealed evidence of permafrost and liquid water in the near subsurface of Mars [1], as well as methane (CH_4) within the Martian atmosphere [2, 3]. On Earth, atmospheric methane is primarily of biological origin. As such, it is possible that the methane on Mars may be evidence of biological organisms such as methanogens. Methanogens are anaerobic microorganisms from the domain Archaea. Many consume hydrogen (H_2) and carbon dioxide (CO_2) to produce CH_4.

Mars experiences wide temperature variations, often ranging from just above freezing (0 °C) to -80 °C and lower, over just one martian sol. The experiment described here exposed four species of methanogens *(Methanothermobacter wolfeii, Methanosarcina barkeri, Methanobacterium formicicum, and Methanococcus maripaludis)* to temperatures of 4°C, -15°C, and -80°C to determine their ability to withstand extremes in temperature.

Methods: Four species of methanogens were initially grown in their own respective anaerobic media [4]. For each of the three temperatures (4 °C, -15 °C, and -80 °C), there were three replicates per species, for a total of 36 tubes. Each tube was inoculated with 0.5 ml of pre-existing culture from the same inoculum. The tubes were then pressurized with 200 kPa H_2 and placed at the organisms' respective growth temperatures for initial growth (*M. wolfeii*: 55 °C; *M. formicicum* and *M. barkeri*: 37 °C; *M. maripaludis*: 24 °C). After one week, the tubes were then placed at one of the three temperatures (4 °C, -15 °C, and -80 °C) for a duration of three months. After three months, the tubes were thawed at room temperature for one week, then returned to their respective incubation temperatures. The tubes were then monitored for methane production over time using gas chromatography.

Results: Cultures of *M. wolfeii* did not survive the prolonged freezing at any temperature (i.e. no methane was produced in any replicate following exposure). Methane production was measured for tubes at -15 °C for both *M. barkeri* and *M. maripaludis* although growth was slow and minimal (Fig. 1). *M. formicicum* survived all temperature trials with significant growth (Fig. 2).

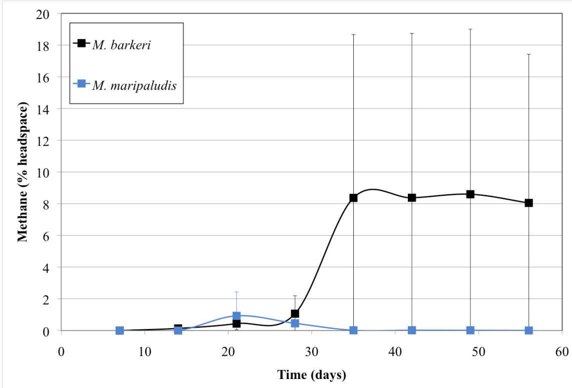

Figure 1. Average methane production (% headspace) for *M. barkeri* (black) and *M. maripaludis* (blue) following incubation at -15 °C for three months.

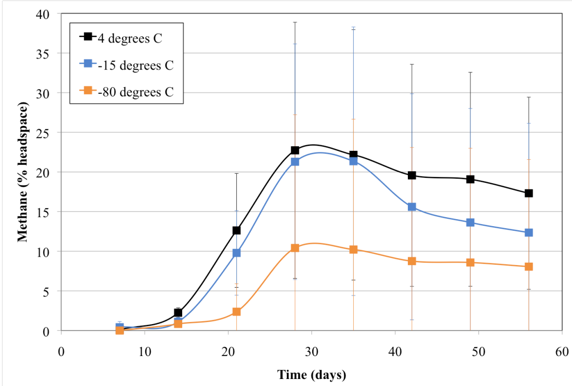

Figure 2. Average methane production (% headspace) for *M. formicicum* following incubation at 4 °C (black), -15 °C (blue) or -80 °C (orange) for three months.

Discussion/Conclusions: The presence of methane produced by the cultures and the increase in methane over time is evidence of methanogen survivability under simulated martian temperatures. Future experiments will test the four species' resilience during freeze/thaw cycles, similar to those seen on Mars (cycling between 4 °C and -80 °C over one day).

References: [1] Mumma, M. J., et al. (2009) *Science*, *323*(5917), 1041-1045. [2] Ojha, L., et al. (2015) *Nature Geoscience 8*(11), 829-832. [3] Webster, C. R., et al. (2015) *Science 347*(6220), 415-417. [4] Kendrick, M. G., and Kral, T. A. (2006) *Astrobiology* 6(4), 546-551.