Methanogenic Archaea as Ideal Candidates for Life on Mars. R. L. Mickol[1], W. H. Waddell[2], and T. A. Kral[1,3], [1]Arkansas Center for Space and Planetary Sciences, University of Arkansas, Fayetteville, Arkansas, 72701, USA, [rmickol@uark.edu], [2]Dept. of Health, Human Performance, and Recreation, University of Arkansas, Fayetteville, Arkansas, 72701, USA, [3]Dept. of Biological Sciences, University of Arkansas, Fayetteville, Arkansas, 72701, USA.

Introduction: The discovery of methane in the martian atmosphere [1, 2] has fueled the study of methanogens as ideal candidates for life on Mars. Methanogens are chemoautotrophs from the domain Archaea that use hydrogen (H_2) as an energy source and carbon dioxide (CO_2) as a carbon source to produce methane (CH_4). Methanogens are ideal candidates for life on Mars because they are anaerobic, do not require organic nutrients and are non-photosynthetic, indicating they could exist in sub-surface environments. Our lab has studied methanogens as models for life on Mars for the past 21 years [3, 4]. Here we present further evidence for methanogen growth and survival under martian conditions.

Methods: The four species of methanogen tested include *Methanothermobacter wolfeii, Methanosarcina barkeri, Methanobacterium formicicum* and *Methanococcus maripaludis*. All methanogens were initially grown in their respective anaerobic growth media. Growth was monitored over time by methane production measured via gas chromatography.

Low temperature (freeze/thaw): *M. wolfeii* and *M. formicicum* were 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 subjected to freeze/thaw cycles between the organisms' ideal growth temperatures (37 °C, *M. formicicum*; 55 °C, *M. wolfeii*) and -80 °C.

Low pressure: Experiments were conducted in the Pegasus Planetary Simulation Chamber (PPSC). All four methanogens were placed into the PPSC and the chamber was evacuated, filled with H_2/CO_2 gas and then maintained at the desired pressure (133-143 mbar, 67-72 mbar, 33-38 mbar, 7-20 mbar, 6-10 mbar) for the duration of the experiments. Following the low-pressure exposure, the methanogens were transferred to new media and incubated at their respective growth temperatures (37 °C, *M. barkeri*; 24 °C, *M. maripaludis*) to test for survival.

Mojave Mars Simulant: All four methanogen species were inoculated into tubes containing fresh media and 10 g sterile Mojave Mars Simulant (MMS).

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 eight conditions consisting of altered MSH medium [5] in order to determine the minimum requirements 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.

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 (below 4 °C), however cultures rebounded after being placed at either room or incubation temperature.

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 was evaporation of the liquid media.

Mojave Mars Simulant: Growth was similar for *M. wolfeii* and *M. formicicum* in both the presence and absence of MMS. Methane production by *M. maripaludis* was significantly hindered in the presence of MMS (compared to normal media). In contrast, *M. barkeri* 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 w/o montmorillonite).

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 [6]. Future experiments will examine the effects of simultaneous multiple factors. In addition, these experiments all assume liquid water is available for metabolism, at least within the near subsurface.

Acknowledgements: R. Mickol was supported by NASA Astrobiology: Exobiology and Evolutionary Biology Program, #NNX12AD90G.

References: [1] Mumma, M. J. et al. (2009) Science, 323, 1041-1045. [2] Webster, C. R. et al. (2015) Science 347: 415-417. [3] Kral, T. A. et al. (2011) *Planet. Sp. Sci.* 59, 264-270. [4] Kral, T. A. and Altheide, S. T. (2013) *Planet. Sp. Sci*., 89, 167-171. [5] Ni, S. and Boone, D. R. (1991) *Int. J. Sys. Bact.,* 41, 410-416. [6] Schuerger, A. C. et al. (2012) *Astrobiology*, 13, 115-131.