Heat Tolerance of *Methanosarcina barkeri* and Survival Below the Surface of Mars. M. P. Plumley[1], R. L. Mickol[2], and T. A. Kral[1,2], [1]Dept. of Biological Sciences, SCEN 601, U. of Arkansas, Fayetteville, AR, 72701 [mpplumle@uark.edu], [2]Arkansas Center for Space and Planetary Sciences, 332 N. Arkansas Ave. U. of Arkansas, Fayetteville, AR, 72701.

Introduction: Methanogens are anaerobic microorganisms that generate methane via methanogenesis as a metabolic byproduct. In methanogenesis, hydrogen (H_2) gas is used to reduce carbon dioxide (CO_2) to produce methane (CH_4). In the presence of sufficient amounts of H_2 and CO_2, this process provides enough energy to support the growth of methanogens [1]. There is a lot of information about the growth and survival of methanogens on Earth, but what is unknown is whether or not methanogens can survive and adapt to conditions outside of their optimal growing conditions. The abundance of CO_2 in the martian atmosphere contributes to a potential carbon source and the suspected presence of H_2 gas below the surface supplies a potential energy source for methanogen growth [2]. The detection of methane in the atmosphere of Mars spurred excitement in the field of astrobiology, suggestive of methanogens as a likely source of the methane as well as the possibility of life surviving below the surface of Mars [3].

Methods: This research explores the heat tolerance of one methanogen species: *Methanosarcina barkeri*. Standard anaerobic liquid media were prepared and 10 mL were dispensed into separate test tubes. After sterilization via autoclave, 2.5% sodium sulfide (Na_2S) was dispensed into the test tubes (~125 uL) to remove any residual oxygen. Next, 0.5 mL culture was transferred to each test tube. The tubes were then pressurized with 200 kPa H_2 gas and incubated at the methanogen's respective growth temperatures (37 °C). After incubation for eight weeks, a gas chromatograph was used to measure the production of methane that occurred without exposure to heat. Each week (after the initial eight week incubation), methane production was measured and each test tube was introduced into a pot of boiling water (100 °C) for a specified time interval (e.g. 5 s, 15 s, 30 s, and 60 s). The tubes were removed from the boiling water and placed back at their incubation temperatures for one week before being subjected to the boiling water again. A control tube containing *M. barkeri* was also incubated, but was not exposed to the boiling water treatment.

Results: Exposure to 100 °C had no deleterious effect on the methane production of *M. barkeri* and may have enhanced growth (Fig. 1). Exposures for time intervals greater than 15 s resulted in a decrease in methane production (Fig. 1).

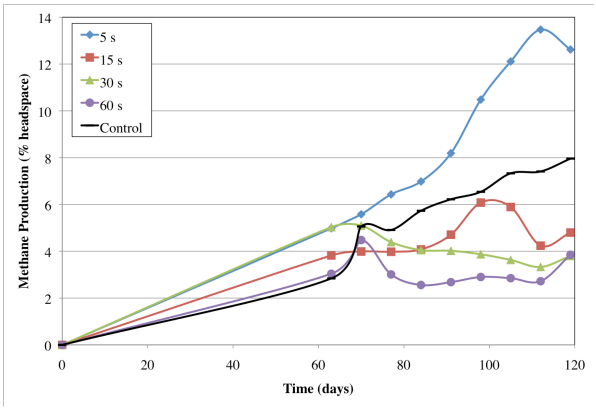

Fig. 1. Methane produced by *M. barkeri* over time. Tubes were exposed to heat (100 °C) at 60 days, then each week thereafter.

Discussion/Conclusion: Exposure to boiling water had varying effects on the methane production by *M. barkeri*, as compared to the control tube. Exposure intervals of 5 s may have promoted growth (methane production). Future work will modify experiments for longer exposure lengths, utilize additional methanogens, and provide more replicates for each time interval.

References: [1] Slonczewski J, Foster J. (2009) *Microbiology*. New York: W.W. Norton & Co. [2] Kral T. A., et al. (2014) *PSS* 101, 181-185. [3] Buford P. P. (2010) *PSS* 58, 1199-1206.