Survival of cells on Mars: exposing methanogens to magnesium perchlorate. M. V. Smith[1], R. L. Mickol[2], and T. A. Kral[2,3], [1]J. William Fulbright College of Arts and Sciences, University of Arkansas, Fayetteville, AR, [mvs003@uark.edu]; [2]Arkansas Center for Space and Planetary Sciences, University of Arkansas, Fayetteville, AR; [3]Dept. of Biological Sciences, University of Arkansas, Fayetteville, Arkansas, 72701, USA.

Introduction: Mars is the most thoroughly explored planet apart from Earth. The fourth planet from the sun is about half the radius and only 11 percent of the mass of Earth. The atmosphere of Mars is thin and composed of mostly carbon dioxide with an average air pressure equal to that of 24 miles above sea level of our home planet [1]. Giovanni Schiaparelli, Italian astronomer in 1877, was the first to see lines through a telescope on the surface of Mars which he termed canali, or channels, which suggested the presence of life [1]. In 2004, rovers Spirit and Opportunity examined martian rocks and concluded that liquid water once existed on the surface of the red planet [1]. According to NASA, the presence of water on Mars is key to finding microscopic organisms that could have developed there [2]. Our laboratory has recently succeeded in growing certain methanogens in the presence of relatively low concentrations of perchlorate salt solutions [3]. Additionally, some of these methanogens were adapted to grow in higher concentrations. Hence, this research project aims to discover if cells can survive after desiccation and exposure to very highly concentrated solutions of magnesium perchlorate [$Mg(ClO_4)_2$] for varying time periods. Understanding the survival and growth of methanogens under martian conditions could further the understanding of the possibility of life on Mars.

Methods: Four species of methanogen were grown in 10 mL of their respective anaerobic growth medium [3]: *Methanothermobacter wolfeii* (55 °C, MM medium), *Methanosarcina barkeri* (37 °C, MS medium), *Methanobacterium formicicum* (37 °C, MSF medium), and *Methanococcus maripaludis* (24 °C, MSH medium). The media were prepared anaerobically and sterilized via autoclave [3]. A 2.5% sodium sulfide (Na_2S) solution was dispensed into each test tube (~125 µL per 10 mL medium) followed by addition of 0.5 mL methanogen cultures. The tubes were pressurized with H_2 gas and then placed at the organisms' respective incubation temperatures (see above). For each experiment, 2-week-old 10 mL stock cultures (as prepared above) were transferred to individual centrifuge tubes and centrifiuged for 20 minutes at 5000 rpm. After centrifugation, the supernatant was poured off and 10 mL of sterile bicarbonate buffer containing 2.5% Na_2S were added to each centrifuge tube and the tubes were centrifuged again. This process was repeated three times to remove residual media [4]. Next, 1 mL of the sterile buffer+Na_2S solution was added to each centrifuge tube, the cell pellet was dislodged, and the 1 mL cells+buffer was transferred to a microcentrifuge tube. The microcentrifuge tubes were centrifuged for 10 minutes, the supernatant was poured off, and the tubes were centrifuged again. The microcentrifuge tubes were then placed in a Coy anaerobic chamber with drierite ($CaSO_4$) overnight to desiccate. The next day, 1 mL 10% $Mg(ClO_4)_2$ was dispensed into each tube. The cells were exposed to the concentrated brine for one hour, after which the brine was poured off. Next, 1 mL of sterile buffer +Na_2S was added to the microcentrifuge tubes and the cells were resuspended. The cell suspension was then transferred to a test tube containing 10 mL of fresh medium, and the tubes were incubated at the organisms' respective growth temperatures. The cultures were then tested for methane production over time.

Results: All four methanogens produced significant amounts of methane following desiccation and one hour exposure to 10% $Mg(ClO_4)_2$ (Fig. 1).

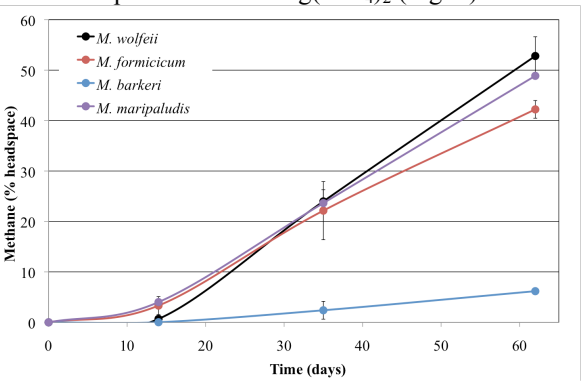

Figure 1. Average methane production (% headspace) by four methanogens following desiccation and exposure to 10% $Mg(ClO_4)_2$ for one hour.

Discussion: The results show each species produced a significant amount of methane following one hour of exposure to 10% $Mg(ClO_4)_2$. This means the cells were able to grow fairly consistently for at least 62 days after brine exposure, as evidenced by the continuing increase in methane (Fig. 1). Future work will test cell exposure to different brines and utilize different exposure time intervals.

References: [1] Trefil, J. (2012) National Geographic, 91-97. [2] NASA: Mars Exploration. Overview. http://mars.nasa.gov. [3] Kral, T. A., et al. (2016) *PSS,* 120, 87-95. [4] McAllister, S. A. and Kral, T. A. (2006) *Astrobiology*, 6(6), 819-823.