SURVIVAL OF METHANOGENIC ARCHAEA AT FREEZING TEMPERATURES. Y. A. Takagi[1], R. L. Mickol[2], Dr. T. A. Kral[2], [1]Oberlin College; ytakagi@oberlin.edu, [2]University of Arkansas

Introduction: Methanogenic archaea are strict anaerobes that can use H_2 and CO_2 as their sole energy and carbon source, respectively, making them good analogues for potential life on Mars [1]. Previous studies have examined the growth of methanogens under various parameters at Mars-like conditions including desiccation, low pressure, starvation, freezing temperatures, and exposure to mars regolith analogues [2,3]. Temperatures on Mars diurnally fluctuate between -80ºC and 20ºC [4]. In order to examine the effects of freezing temperatures on methanogens more closely, three experiments were conducted: growth at cold temperatures, survival of Martian temperature cycles, and effects of glass particles on survival at freezing temperatures.

Shared Methods: Four species of methanogen were used: Methanothermobacter wolfeii (55ºC, MM growth medium), Methanosarcina barkeri (37ºC, MS growth medium), Methanobacterium formicicum (37ºC, MSF growth medium) (Fig. 1), Methanococcus maripaludis (25ºC, MSH growth medium)**.** Cultures were grown in anaerobic test tubes in 10 mL of media under an atmosphere (headspace) of H_2 and CO_2. The headspace was initially pressurized to 180 kPa. Tubes were inoculated with 0.5 mL media from a stock culture. Gas chromatography (GC) was used to determine the methane concentration of the headspace, which was used as a proxy for metabolism/growth. Absorption spectrometry at 600 nm was used to measure optical density, which was used as a proxy for biomass.

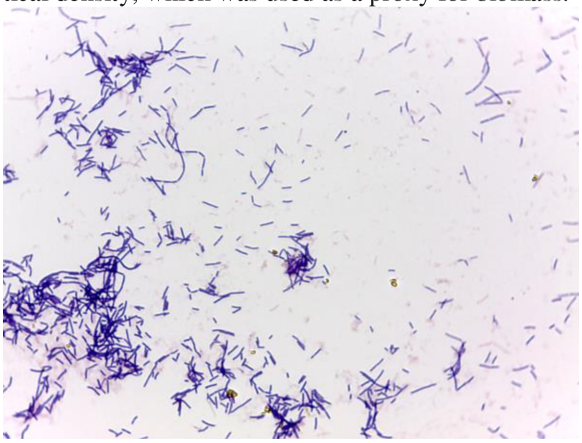

Figure 1. M. formicicum Gram stained and imaged with a Leica DM750 Microscope and ICC50 camera.

Growth at Cold Temperatures

Additional Methods: Four tubes of each species were grown at 4ºC, and three tubes at 22ºC. GC and Absorbance measurements were taken every two weeks.

Results:

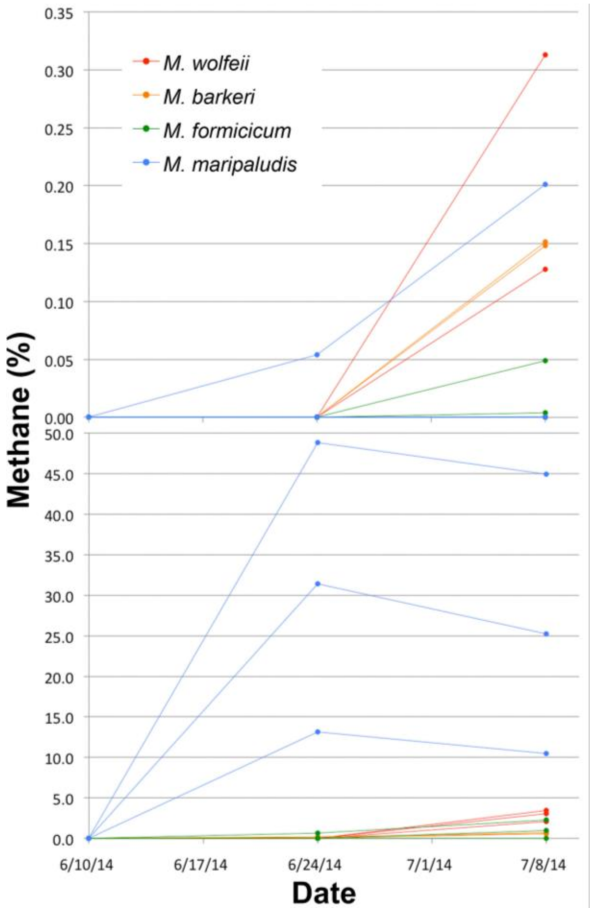

Figure 2. Growth of methanogens at cold temperatures over time measured via proxy by methane percentage of the headspace. (Top) 4ºC. Four replicates per species. (Bottom) 22ºC. Three replicates per species.

Discussion/Conclusions: All species appear to be viable at both 22ºC and 4ºC (Fig. 2). M. maripaludis grew best at 22ºC, as expected. It is surprising that M. wolfeii grows better than the other species at low temperatures. It is possible that the same mechanisms that allow M. wolfeii to thrive at high temperatures (such as the presence of chaperone proteins [5]) aid in cold temperatures.

Survival of Martian Temperature Cycles

Additional Methods: Following inoculation, cultures were grown for five days at incubation temperatures, and six days at 22ºC. Cultures were then exposed to temperature cycles stepping between fixed temperature freezers of 22ºC, 4ºC, -15ºC, and -80ºC. Three tubes of each species were subjected to bi-diurnal temperature cycles for 12 days. Four tubes of each species were subjected to diurnal temperature cycles for 10 days. Cultures were then returned to incubation temperature. GC and Absorbance measurements were

taken periodically throughout the temperature cycling and recovery period.

Results: Several tubes were lost due to explosion during the experiment. A few tubes were discarded due to exposure to oxygen.

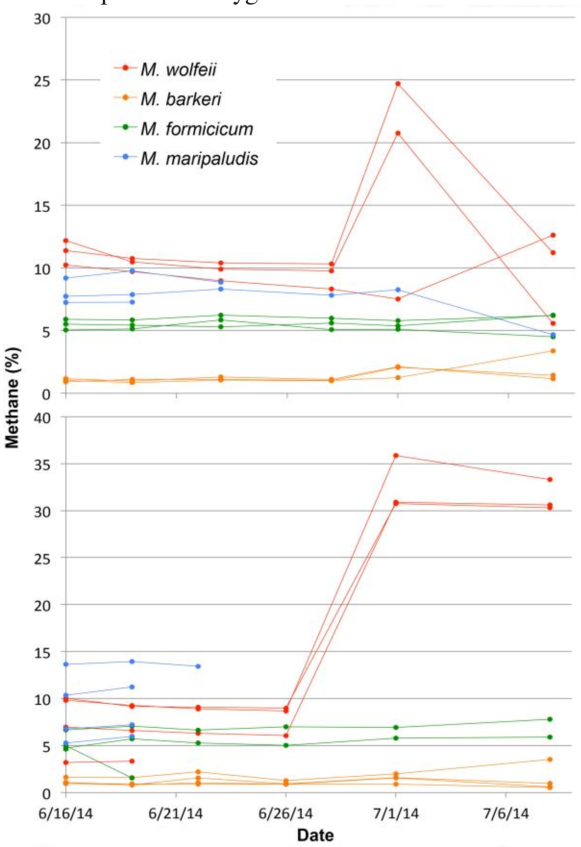

Figure 3. Methane fraction over time while being exposed to Mars-like temperature cycles. (Top) Bi-diurnal temperature cycles. Three replicates per species. Cultures returned to incubation temperatures on 6/28/14. (Bottom) Diurnal temperature cycles. Four replicates per species. Cultures returned to incubation temperatures on 6/26/14.

Discussion/Conclusions: No growth occurred during the temperature cycling (Fig. 3). M. wolfeii and M. barkeri both recovered metabolic activity after completion of the cycles, but subsequently ceased activity due to dormancy, damage, or death.

Effects of Glass Particles on Survival at Freezing Temperatures

Methods: Three tubes each of each species were prepared with media including no glass, glass wool, glass beads (2 mm diameter), and crushed glass shards/dust. Cultures were grown at incubation temperatures for 6 to 14 days, subjected to -15ºC for 1 day, then returned to incubation temperatures. GC measurements were taken periodically before and after the freeze event.

Results:

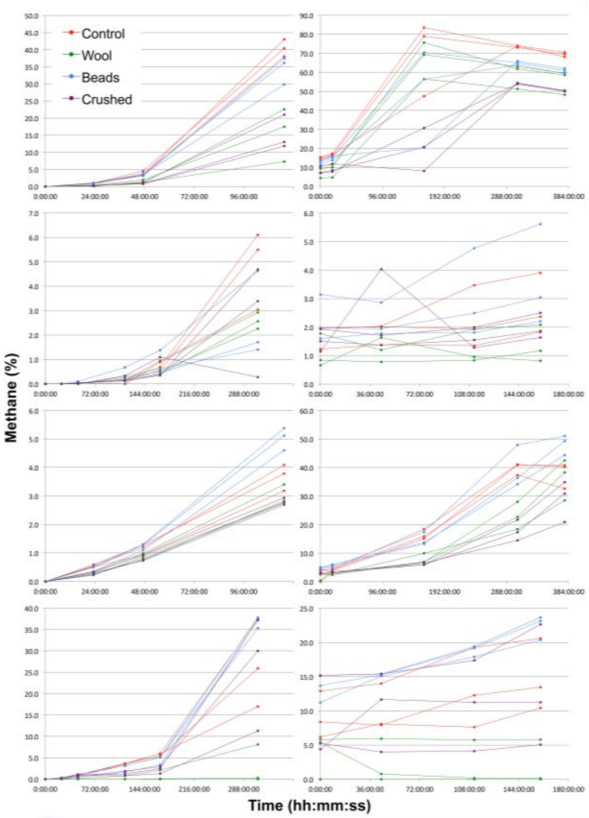

Figure 4. Methane fraction over time for cultures with media containing glass. Three replicates per glass-type per species. Left column: data before freeze-event. Right column data after freeze event. Rows, top to bottom: M. wolfeii, M. barkeri, M. formicicum, M. maripaludis.

Discussion/Conclusions: Exposure to different glass particles may have an effect on growth rate for some organisms (Fig. 4). As glass beads assist organisms in surviving desiccation [3], it was hypothesized that glass particles may also assist organisms in surviving freezing temperatures. However, the results are unclear. Organisms may enter a state of metabolic maintenance (but not active growth) following exposure to freezing temperatures, obfuscating the validity of our exponential growth model.

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References: [1] Morozova, D. et al. (2007) Origins Life Evol. B. 37 (2), 16-25. [2] Kral, T. A. et al. (2013) Planet Space Sci. 89, 167-171. [3] Morozova, D. et al. (2007) FEMS Microbiol. Ecol. 61 (1), 16-25. [4] Ulrich, R. et al. (2010) Astrobiology 10 (6), 643-650. [5] Clarke, A. (2014) Int. J. Astrobiol. 13 (2), 141-154.