**Survival of Methanogens on Different Martian Regolith Analogs: Implications for Life Detection in Returned Martian Samples.** T. A. Kral[1,2] and T. S. Altheide[2], [1]Dept. of Biological Sciences, University of Arkansas, Fayetteville, AR, [2]Arkansas Center for Space and Planetary Sciences, University of Arkansas, Fayetteville, AR. tkral@uark.edu.

**Introduction:** We have been studying methanogens as models for life on Mars for the past 18 years (1, 2, 3, 4, 5, 6, 7, 8, 9). Methanogens are microorganisms in the domain Archaea that can metabolize $H_2$ as an energy source, $CO_2$ as a carbon source, and produce methane. They are one possible explanation for the methane found in the Martian atmosphere (10, 11, 12). If an organism is to exist in the hostile Martian environment, it must be able to deal with a number of relatively extreme factors. Three of those factors are limited availability of liquid water, low pressure, and minimal nutrients. Here we report on research designed to determine if certain species of methanogens can survive desiccation at Mars surface pressure of 6 mbar, in different Martian regolith analogs, for both 90 and 120 days.

**Methods:** The low-pressure desiccation experiments were performed in the Pegasus Chamber, located at the Arkansas Center for Space and Planetary Sciences, University of Arkansas, Fayetteville. The methanogens tested, *Methanosarcina barkeri, Methanobacterium formicicum, Methanothermobacter wolfeii and Methanococcus maripaludis,* were grown in their respective growth media in anaerobic culture tubes. Following five days of growth, cultures were centrifuged followed by suspension of the cell pellets in 700 uL of sterile buffer containing sodium sulfide (to remove residual molecular oxygen). In a Coy anaerobic chamber, 10 uL of each cell suspension were added to anaerobic culture tubes containing sterile Martian regolith analogs (montmorillonite, nontronite, basalt, jarosite or JSC Mars-1 soil simulant) or glass beads (control). The tubes were removed from the anaerobic chamber and placed into the Pegasus chamber. The chamber was sealed and evacuated down to 6 mbar, resulting in desiccation of all of the cultures. Following 90 days and 120 days (separate experiments), the tubes were removed from the chamber, rehydrated with ideal growth media, and placed under ideal growth temperatures for the respective methanogens. Additionally, in the 90-day experiment, some of the rehydrated cells from the glass beads were transferred to regolith analogs, and some of the rehydrated cells from regolith analogs were transferred to fresh medium without regolith analogs. At regular time intervals, headspace gas samples were removed and analyzed for methane using gas chromatography.

**Results and Discussion:** All three organisms that were placed on glass beads demonstrated substantial methane production with time (50 percent or greater of the headspace gas) following both 90 (Table 1) and 120 (Table 2) days of desiccation. *M. barkeri* survived on multiple Martian analogs (JSC Mars-1, montmorillonite and basalt) following the two desiccation periods. Also, some *M. wolfeii* and *M. barkeri* cells transferred to and from regolith analogs demonstrated survival. No organism survived on the nontronite. Based on the three factors tested here, the results would seem to indicate that some methanogens may be able to survive and possibly thrive on Mars. If methanogens inhabit the regolith of Mars, and if some are able to survive desiccation at low pressure as seen here, then returned samples of Martian regolith may yield viable cells.

| | Glass | Substrate* | Glass→Substrate | Substrate→Medium |
|---|---|---|---|---|
| *M. wolfeii* | +++ | - | + (SS & Basalt) | + (Clay & Basalt) |
| *M. barkeri* | +++ | ++ (SS & Clay) | + (Clay & Basalt) | ++ (SS & Clay) |
| *M. formicicum* | ++ | - | - | - |
| *M. maripaludis* | - | - | - | - |

*JSC Mars-1 (SS)
Clay (Montmorillonite)
Basalt

Table 1. Methane production by *Methanothermobacter wolfeii, Methanosarcina barkeri, Methanobacterium formicicum* and *Methanococcus maripaludis* following desiccation at 6 mbar for 90 days.

| | Glass Beads | Ch. Basalt | Gr. Basalt | Ch. Jarosite | Gr. Jarosite |
|---|---|---|---|---|---|
| *M. wolfeii* | +++ | - | - | - | - |
| *M. barkeri* | +++ | ++ | ++ | ++ | - |
| *M. formicicum* | ++ | - | - | ++ | - |
| *M. maripaludis* | - | - | - | - | - |

Ch. = Chunk
Gr. = Ground

Table 2. Methane production by *Methanothermobacter wolfeii, Methanosarcina barkeri, Methanobacterium formicicum* and *Methanococcus maripaludis* following desiccation at 6 mbar for 120 days.

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