Survival of Methanogens Exposed to Diurnal Freeze/Thaw Cycles. R. L. Mickol[1], Y. A. Takagi[2], and T. A. Kral[1,3], [1]Arkansas Center for Space and Planetary Sciences, University of Arkansas, Fayetteville, AR; [2]Dept. of Biology, Oberlin College, Oberlin, OH; [3]Dept. of Biological Sciences, University of Arkansas, Fayetteville, AR; [rmickol@uark.edu]

Introduction: Methanogens are microorganisms in the domain Archaea that utilize hydrogen (H_2) and carbon dioxide (CO_2) to produce methane (CH_4). The discovery of methane in the martian atmosphere [1, 2] reinforces the study of methanogens as candidates for life on Mars.

Mars experiences wide temperature variations over one sol, often ranging from temperatures just above freezing (0 °C) to -80 °C and lower [3]. Any microorganisms that could potentially inhabit Mars would at least need to be able to survive these temperatures, and also make use of any available liquid water or temporary increases in temperature in order to metabolize. The experiments described here expose four methanogen species (*Methanothermobacter wolfeii*, *Methanobacterium formicicum*, *Methanosarcina barkeri*, *Methanococcus maripaludis*) to diurnal temperature changes between -80 °C and 22 °C.

Methods: Two separate experiments were performed: in the first experiment, a diurnal (24-h) temperature cycle was used; the second experiment used a 48-h cycle (Table 1).

Table 1. Temperature Cycling for Two Experiments

Expt. 1: 24-h Cycling		Expt. 2: 48-h Cycling	
-80 °C to -15 °C	2 hours	-80 °C to -15 °C	5 hours
-15 °C to 4 °C	1 hour	-15 °C to 4 °C	4 hours
4 °C to 22 °C	2 hours	4 °C to 22 °C	2 hours
22 °C to 4 °C	2 hours	22 °C	13 hours
4 °C to -15 °C	2 hours	22 °C to 4 °C	5 hours
-15 °C to -80 °C	2 hours	4 °C to -15 °C	4 hours
-80 °C	13 hours	-15 °C to -80 °C	3 hours
		-80 °C	12 hours

Each methanogen was initially grown in its respective anaerobic growth medium. An aliquot of 0.5 mL of culture was inoculated into 10 mL of fresh medium for experimentation. Tubes were pressurized with 1.8 bar H_2 and incubated at a temperature within each organism's growth range (*M. wolfeii: 55* °C*; M. formicicum*, *M. barkeri*: 37 °C; *M. maripaludis: 24* °C) for 5 days, then incubated at 22 °C for an additional 6 days. Cultures were then exposed to a specific freeze/thaw cycle (Table 1) for 10 days (Expt. 1; n = 4) or 12 days (Expt. 2; n = 3). After the exposure period, cultures were incubated at their respective growth temperatures. Growth was monitored by methane production via gas chromatography and optical density.

In a third experiment, cultures were prepared as above and were then kept at 4 °C (n = 4) or 22 °C (n = 3) and monitored for growth.

Results: All four methanogen species (except *M. maripaludis*, see Discussion) survived both the diurnal (Fig. 1) and 48-h temperature cycles. Methane production was also possible for all four methanogens at both 4 °C and 22 °C, although growth was much slower at the lower temperatures, as expected.

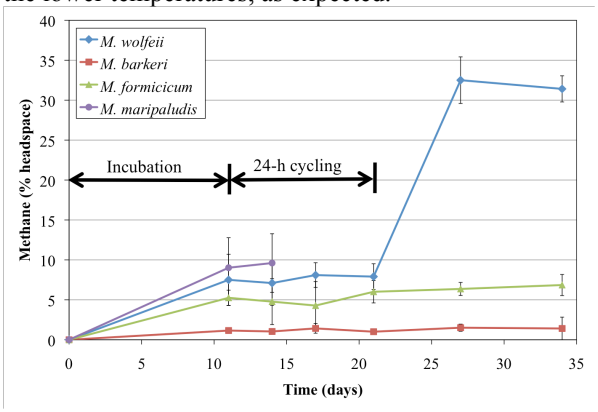

Figure 1. Methane production (% headspace) by four methanogens following an initial incubation period and exposure to daily temperature changes between -80 °C and 22 °C. Error bars indicate one standard deviation.

Discussion: Non-psychrophilic methanogens are capable of metabolism at low temperature and can also survive extreme daily temperature changes, similar to those on Mars. For both temperature cycling experiments, some tubes "exploded" during the cycling period and were excluded from additional growth monitoring (Fig. 1, *M. maripaludis*). Average methane production was similar for three of the four methanogens (*M. barkeri, M. formicicum, M. maripaludis*) for both the 24-h and 48-h cycles. Interestingly, the methanogen with the highest growth temperature (*M. wolfeii*, 55 °C) produced greater amounts of methane following the 24-h cycling as compared to the 48-h cycling.

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