Survival of Methanogens Exposed to Diurnal Freeze/Thaw Cycles

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INTRODUCTION

Mars experiences wide temperature variations over one sol, often ranging from temperatures just above freezing (0 °C) to -80 °C and lower [1]. 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. 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 [2, 3] reinforces the study of methanogens as candidates for life on Mars. 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

- Methanogens were grown in their respective anaerobic growth media.
- Aliquots of 0.5 mL of culture were inoculated into 10 mL of fresh media for experimentation.
- Culture tubes were pressurized with 1.8 bar H_2 and incubated at the organisms' ideal growth temperatures (*M. wolfeii: 55* °C*; M. formicicum*, *M. barkeri*: 37 °C; *M. maripaludis: 24* °C) for five days, then incubated at 24 °C for an additional 6 days.
- Cultures were exposed to a specific freeze/thaw cycle (Table 1) for 10 days (Expt. 1; n = 4) or 12 days (Expt. 2; n = 3).
- After exposure, cultures were incubated at the organisms' 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 = 4) and monitored for growth.

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

RESULTS

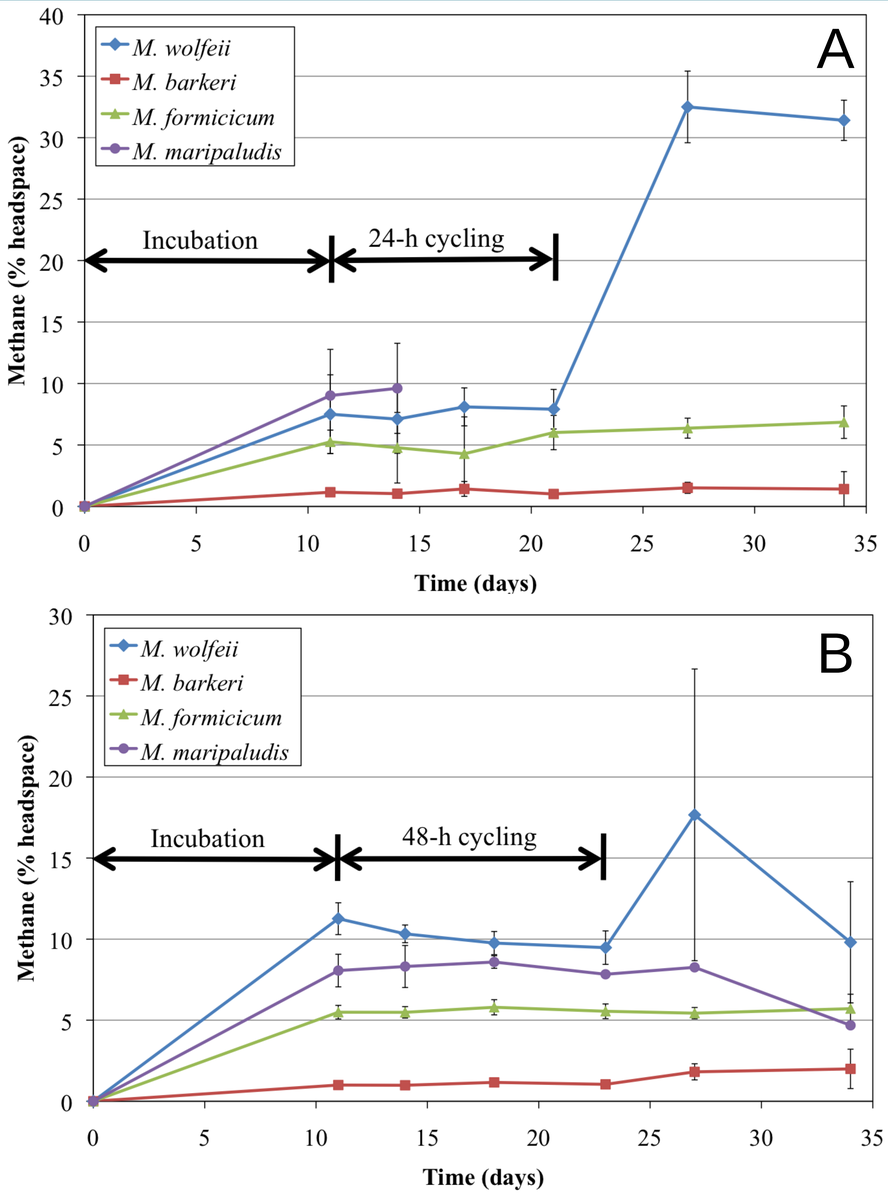

Figure 1 (left). Methane production (% headspace) by four methanogen species (*Methanothermobacter wolfeii*, *Methanobacterium formicicum*, *Methanosarcina barkeri*, *Methanococcus maripaludis*) following an initial incubation period and exposure to diurnal (**A**) or 48-hour (**B**) temperature cycling between -80 °C and 22 °C (Table 1). Error bars indicate ± one standard deviation.

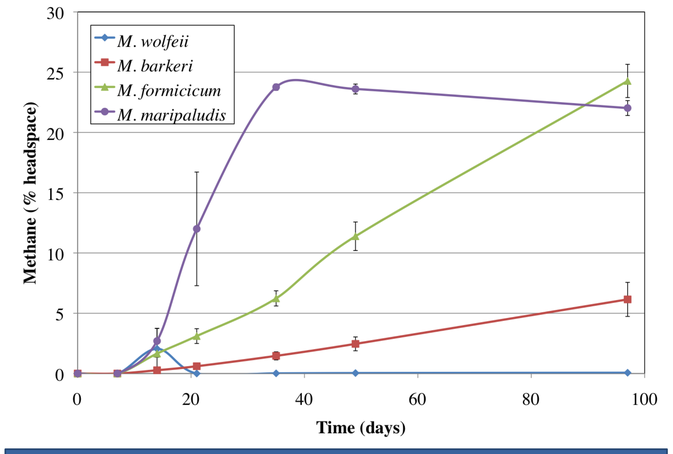

Figure 2 (above). Methane production (% headspace) by four methanogen species (*Methanothermobacter wolfeii*, *Methanobacterium formicicum*, *Methanosarcina barkeri*, *Methanococcus maripaludis*) incubated at 22 °C. Error bars indicate ± one standard deviation.

DISCUSSION

Non-psychrophilic methanogens are capable of metabolism at room temperature (22 °C) and can also survive extreme daily temperature changes between -80 °C and 22 °C, similar to those on Mars. However, incubation at 4 °C proved inhibitory for all four species (data not shown). For both temperature-cycling experiments, some tubes "exploded" during the cycling period and were excluded from additional growth monitoring (Fig. 1A, *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. Additionally, 22 °C proved inhibitory to methane production by *M. wolfeii*, although a slight amount of methane (~2%) was produced within 14 days.

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