Methanogens as Models for Life on Mars

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BACKGROUND

- Methane on Mars fuels the study of methanogens as models for life on Mars [1-6]
- Methanogens are chemoautotrophs from the domain Archaea that produce methane from H_2 and CO_2
- Four methanogen species (*Methanobacterium formicicum*, *Methanosarcina barkeri*, *Methanothermobacter wolfeii*, *Methanococcus maripaludis*) were tested for their ability to survive various martian conditions

METHODS

- 50 mL of 4 types of methanogen growth medium (MM, MS, MSH, MSF) prepared [7], with 10 mL of each medium added to each of 5 test tubes
- Sterile 2.5% Na_2S solution added to tubes following sterilization via autoclave
- Each tube inoculated with 0.5 mL of the corresponding methanogen (MM: *M. wolfeii*; MS: *M. barkeri*; MSH: *M. maripaludis*; MSF: *M. formicicum*)
- Tubes pressurized with H_2, incubated at ideal growth temps, and tested for methane via gas chromatograph

Low Pressure

- Tubes placed inside Pegasus Planetary Simulation Chamber [8]
- Chamber evacuated to 1 mbar, filled with 80:20 H_2:CO_2 gas to 100 mbar, evacuated to 1 mbar (cycle repeated 3 times), and set at desired pressure
- Tubes punctured after 2 days, with a specialized device, then unpunctured at set time, removed from chamber, transferred to new media and incubated
- Six experiments run: 133 mbar, 67 mbar (2), 33 mbar, 6 mbar, 7 mbar

Low Temperature

- Four sets created with 1.5-10 mL medium and 0-10 g sand or 0-5 g gravel, using *M. wolfeii* (MM medium) or *M. formicicum* (MSF medium)
- Tubes subjected to freeze/thaw cycles between -80°C and 55°C
- Transfers to new media performed at least once during experiment

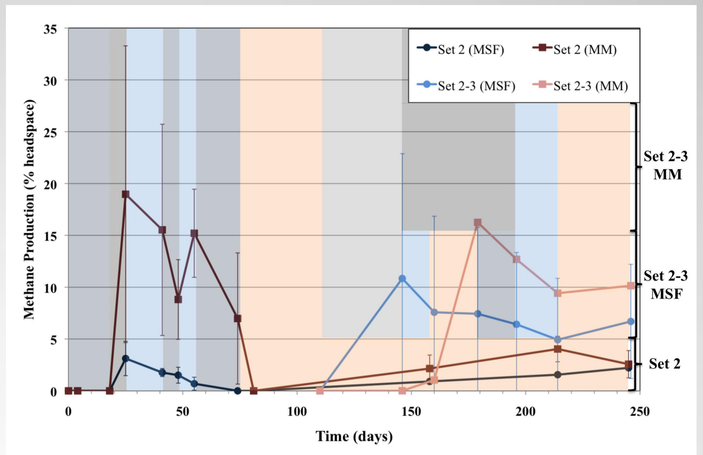
Mojave Mars Simulant (MMS)

- Sets contained fresh media and 10 g MMS, monitored for growth for 140 days

Montmorillonite

- Expt. utilized *M. maripaludis* (MSH medium) only, monitored for 15 days
- 8 conditions of altered medium and montmorillonite: 0.5 g montmorillonite (except control) and 10 mL of MSH medium, buffer, salt solution, or altered MSH medium

RESULTS



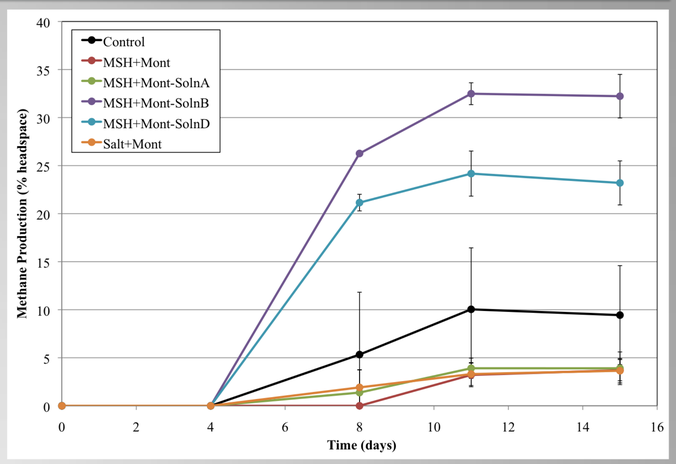


Figure 1. A. Average methane production (% headspace) over 245 days for each of 4 subsets (2 original, 2 transfer) containing *M. formicicum* (MSF) or *M. wolfeii* (MM) in 1.5 mL medium and 5 g sand. Colored columns represent temperature (gray = 37°C, orange = 25°C, dark blue = 4°C, light blue = -15°C). Error bars are one standard deviation. Original tubes kept at 24°C from Day 74 to Day 245. **B.** Average methane production over 15 days for *M. maripaludis* subjected to 8 conditions containing 0.5 g montmorillonite and 10 mL solution. Mont+buffer and MSH+Mont-Salts conditions failed to produce methane.

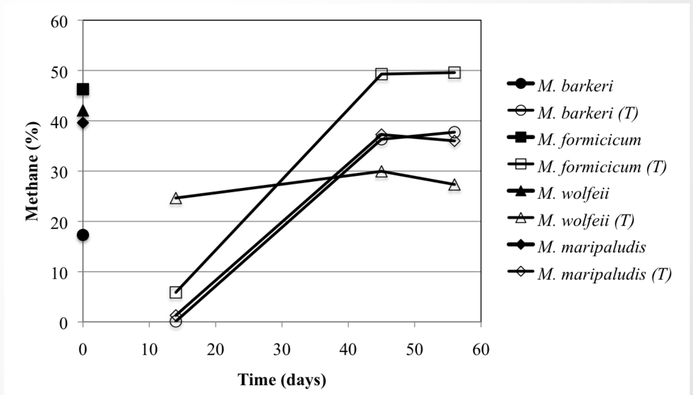


Figure 2. Average methane produced for 4 methanogen strains (*M. barkeri*, *M. formicicum*, *M. wolfeii*, *M. maripaludis*) before/after 3 days at 6-10 mbar. Original tubes placed in chamber on Day 0. Closed symbols are original tubes, open symbols are transfer (T) tubes.

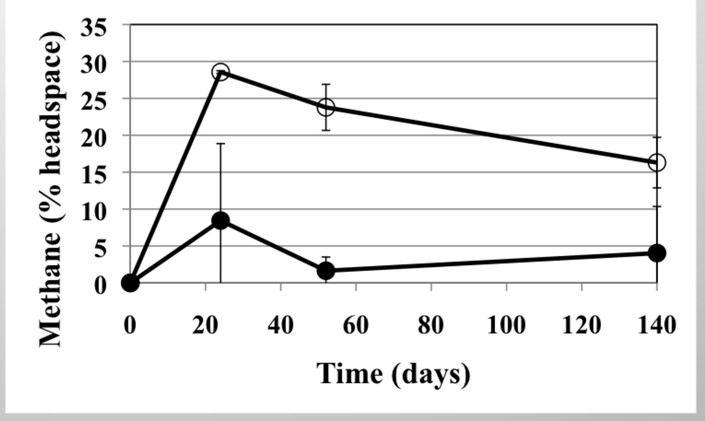


Figure 3. Average methane produced for *M. maripaludis* in 10 mL MSH medium with 10 g Mojave Mars Simulant (closed symbols). Open symbols are tubes without MMS. Error bars are 1 standard deviation.

DISCUSSION/CONCLUSIONS

- Freeze/thaw cycles inhibit methane production but methanogenesis resumes after incubation at 24°C
- Limiting factor is evaporation of liquid media in low pressure experiments, but all 4 methanogens survived exposure
- Growth (methane production) is similar in the presence and absence of Mojave Mars Simulant, except for inhibition of *M. maripaludis* (Fig. 3)
- Growth of *M. maripaludis* enhanced by addition of montmorillonite and deletion of two solutions from standard medium

Acknowledgements

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