Methanogens as Models for Life on Mars
Rebecca L. Mickol¹, W. H. Waddell² and T. A. Král¹,²
¹Arkansas Center for Space and Planetary Sciences, University of Arkansas; rmickol@uark.edu
²Department of Biological Sciences, University of Arkansas

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 H₂ and CO₂
- 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% Na₂S 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 H₂, 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 H₂:CO₂ 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

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

Montmorillonite
- Expt. utilized M. maripaludis (MSH medium) only, monitored for 140 days

Montmorillonite
- 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.

Acknowledgements
The authors would like to acknowledge NASA grant #NNX12AD96G and thank W. Graupner, S. Laird and the Arkansas Center for Space and Planetary Sciences for their facilities and research assistance.

References