CLAY MINERALS: A MARTIAN MICROBE'S FAVORITE SNACK.

P.I. Craig[1], R.L. Mickol[2], C.L. Marnocha[3], T.A. Kral[4,5], [1]Planetary Science Institute, 1700 E. Ft. Lowell Rd, #106, Tucson, AZ, (pcraig@psi.edu). [2]American Society for Engineering Education, Washington, DC. [3]Dept. of Biology, Niagara University, NY. [4]Arkansas Center for Space and Planetary Sciences, University of Arkansas, Fayetteville, AR. [5]Dept. of Biological Sciences, University of Arkansas, Fayetteville, AR.

Introduction: The identification of clay minerals in the oldest (Noachian) surface of Mars suggests the existence of near-neutral liquid water during that era. The most commonly identified clay minerals on Mars to date are Fe/Mg-smectite (nontronite or saponite) and Al-smectite (montmorillonite) [1]. If the martian surface hosted liquid water in the past, there is a possibility that life may have arisen and thrived in these regions. Not only have clay minerals been identified in the martian Noachian terrain, but also in the central mound (Aeolis Mons) of Gale Crater, where the MSL *Curiosity* rover is currently exploring. These clay minerals are part of a strata that represent the geologic and aqueous history of Gale Crater [e.g. 2]. Current mineralogical data from the rover suggests Gale Crater was once a lake [3], lending further credence to the possibility of the existence of past life.

Here, we conducted experiments to test the ability of methane-producing microbes (methanogens) to survive on a Mars-like clay mineral substrate. Furthermore, we monitored methane (CH_4) production as a proxy for metabolism, and analyzed the clay minerals to identify and characterize possible biosignatures left behind.

Methods: Two methanogens, *Methanobacterium formicicum* and *Methanosarcina barkeri*, were tested for their ability to grow in the presence of montmorillonite, a mixture of montmorillonite and nontronite, or a mixture of montmorillonite and kaolinite without the use of additional nutrients. In the first batch of experiments, one gram of montmorillonite was added to each of five test tubes. In the second set, test tubes contained a mixture of 0.5 g each of montmorillonite and nontronite. The third set consisted of tubes with a mixture of 0.5 g each of montmorillonite and kaolinite. Tubes were deoxygenated overnight, then filled with 10 mL bicarbonate buffer. The tubes were sealed with rubber stoppers and crimps, then sterilized via autoclave. Methanogens were aerobically washed [4], then 0.5 mL cells+buffer were inoculated into each tube containing the sterilized clay solutions. The tubes were pressurized with 2 bar H_2, incubated at 37 °C, and monitored over time for CH_4 production. Each set also included negative controls (clay mineral only, no microbes).

After 195 days, the minerals were removed from the tubes and analyzed for the presence of possible biosignatures in the form of mineralogical and/or structural changes using X-ray diffraction (XRD).

Results: *M. barkeri* failed to produce any CH_4 on the montmorillonite-kaolinite mixture, but produced up to 9% CH_4 on montmorillonite+nontronite and 6% CH_4 on montmorillonite alone after 195 days (data not shown). *M. formicicum* produced the most CH_4 on montmorillonite, but was less successful on the mixtures than control cultures (containing no clay minerals and normal anaerobic medium) (Fig. 1).

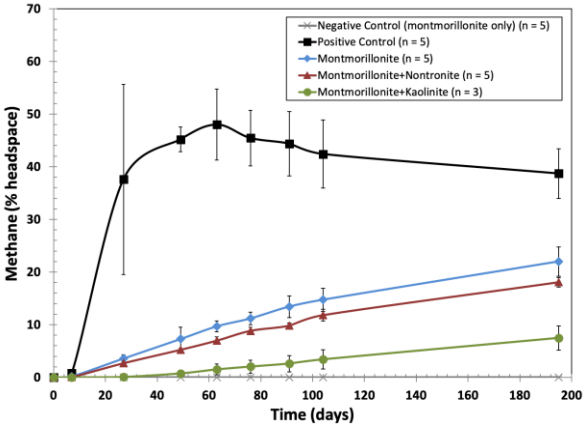

Figure 1: Methane production (% headspace) over time by *M. formicicum* on various clay mineral substrates.

No significant mineralogical changes were observed via XRD in any of the reacted clay minerals. However, detailed analyses of the exact position of the 001 peak, which indicates interlayer spacing, showed slight changes, both increases and decreases, of the interlayer spacing (Figs. 2-4).

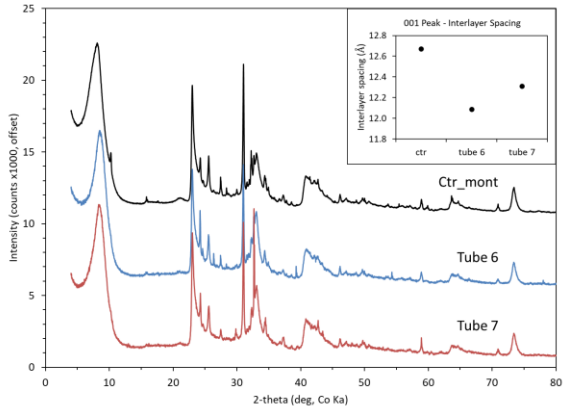

Figure 2: XRD patterns of montmorillonite with *M. formicicum* compared to the negative control. Ctr_mont = control

sample – only clay mineral and buffer, no microbes. Inset: Changes in position of the 001 interlayer peak at low 2-theta angles.

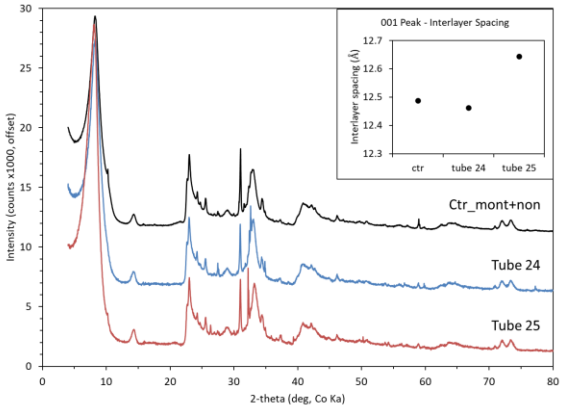

Figure 3: XRD patterns of montmorillonite-nontronite mixture with *M. formicicum* compared to the negative control. Ctr_mont+non = control sample – only clay mineral and buffer, no microbes. Inset: Changes in position of the 001 interlayer peak at low 2-theta angles.

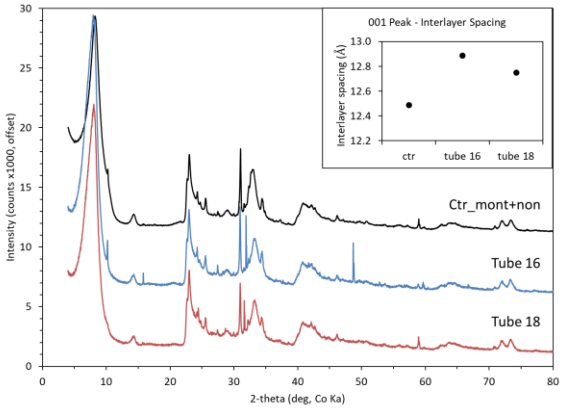

Figure 4: XRD patterns of montmorillonite-nontronite mixture with *M. barkeri* compared to the negative control. Ctr_mont+non = control sample – only clay mineral and buffer, no microbes. Inset: Changes in position of the 001 interlayer peak at low 2-theta angles.

Discussion: These experiments limited micronutrients (e.g. added vitamins and minerals) from the medium in an attempt to determine the ability of two methanogen species to utilize nutrients solely provided by the clay mineral substrate. Of the two species and three substrates tested, *M. formicicum* fared the best on montmorillonite. Although no mineralogical changes were detected via XRD, slight changes in the interlayer spacing of the clay minerals were observed.

In cases where an increase in the interlayer spacing was detected (tube 25, Fig. 3; tubes 16 and 18, Fig. 4), the microbes may have left organic material in the interlayer, expanding it relative to the control sample. Cases where a decrease in the interlayer spacing was detected (tubes 6 and 7, Fig. 2; tube 24, Fig. 3) could suggest the microbes were drawing nutrients from the ions in the interlayer of the clay mineral (e.g. Na, Ca, K, Mg), collapsing it relative to the control. In conjuction with CH_4 production over time, these results appear to indicate that the methanogens were able to utilize nutrients from the clay minerals to support metabolism. This suggests that this sort of nutrient utilization could have taken place, or could still be taking place, on Mars.

Implications for Mars: The presence of clay minerals on Mars and the likely aqueous history of the planet is highly suggestive that life might once have existed there. Currently, the martian subsurface offers the most habitable environment by providing protection from UV radiation [5] and (previously) aqueous clay minerals offer the potential for supplying any past or present microorganisms with micronutrients. These data indicate that some species of methanogen can indeed survive using only clay minerals as a source of nutrients, provided only with an energy source in the form of hydrogen (hypothesized to exist in the subsurface [6,7]) and a carbon source in the form of carbon dioxide (abundant in the martian atmosphere).

Further support for the presence of methanogens on Mars comes from the mandatory byproduct of their metabolism: CH_4. Short-lived increases of atmospheric CH_4 have been detected over Nili Fossae from ground-based telescopes [8] and in Gale Crater by the SAM instrument on the MSL *Curiosity* rover [9,10]. Our data suggest that CH_4 production is possible using clay minerals found on Mars as a substrate, which could potentially be the source of these recently observed CH_4 plumes on Mars.

Future Work: Based on the promising results of previous work [11,12], we plan to implement SEM/EDS analysis to detect and characterize possible micro-scale textural changes in the clay minerals and elemental depletions in the new textures. We also intend to use EGA to characterize the nature of volatiles and organic material in the samples. Finally, we plan to obtain VNIR spectra of each sample to characterize the water content and organic material in the samples.

References: [1] Carter, J., et al., (2015) *Icarus* 248, 373-382. [2] Fraeman, A., et al., (2013) *Geology* 41, 1103-1106. [3] Grotzinger, J., et al., (2015) *Science* 350, 6257. [4] McAllister, S.A., and Kral, T.A., (2006) *Astrobio* 6, 819-823. [5] Cockrell, C., et al., (2000) *Icarus* 146, 343-359. [6] Tate, C., et al., (2018) *Icarus* 299, 513-537. [7] Tarnas, J., et al., (2018) *EPSL* 502, 133-145. [8] Mumma, M., et al., (2009) *Science* 323, 1041-1045. [9] Webster, C., et al., (2015) *Science* 347, 415-417. [10] Webster, C., et al., (2018) *Science* 360, 1093-1096. [11] Mickol, R., et al., (2016) Biosig. Workshop, #2035. [12] Craig, P., et al., (2017) *LPSC XLVIII*, #1997.