GROWTH OF METHANOGENS ON A MARS SOIL SIMULANT. T. A. Kral, C. R. Bekkum, and D. R. Ormond, Arkansas-Oklahoma Center for Space and Planetary Sciences and Department of Biological Sciences, University of Arkansas, Fayetteville, AR, 72701, USA (tkral@uark.edu).

Even though the Viking Lander missions in 1976 found no substantial evidence of life on the surface of Mars [1], investigators continue to believe that life may exist in subsurface habitats. A subsurface source of molecular hydrogen, along with carbon dioxide (which is already abundant in the martian atmosphere), and liquid water, might support the growth of chemoautotrophic methanogenic microorganisms. Because nutrient requirements for many methanogens are so minimal, the possibility exists that methanogenic species can grow in an aqueous environment on Mars in which the soil supplies any additional required nutrients. If growth occurs under such conditions, it is also of interest to determine the minimal amount of water required to sustain growth.

We have designed experiments to address these questions using the JSC Mars-1 simulant [2] as a proxy for martian soil. *Methanosarcina barkeri*, *Methanobacterium wolfei*, and *Methanobacterium formicicum* were grown in standard media, centrifuged, and washed three times with a carbonate buffer solution. The cell pellets were suspended in the same buffer and then varying volumes of each were anaerobically added to sealed tubes, each containing 5 g of Mars soil simulant in a carbon dioxide and hydrogen atmosphere. The total amount of water in each tube varied from less than saturating to standing liquid. Each culture tube was then incubated at the optimal temperature for the respective organism.

We found that the Mars soil simulant contains sufficient nutrients to support growth (as determined by methane production) of all three methanogens [3]. *M. wolfei* shows methane production when 2 ml of cell suspension are added to the 5 g of Mars soil simulant, but grows better when standing liquid is present. *M. barkeri* grows well when 2 to 3 ml are added, and not as well when standing liquid is present. *M. formicicum* prefers more water, but will grow without standing liquid.

In order to exclude the buffer as a source of trace contaminants that might have allowed for the growth observed, methanogenic cells were washed and suspended in the liquid fraction of a Mars soil simulant/de-ionized water mixture. Liquid fractions were prepared by adding varying amounts of Mars soil simulant to de-ionized water and allowing them to mix in a shaking incubator overnight. The liquid fractions were decanted into serum bottles, purged with argon to remove oxygen, and autoclaved. Methanogenic cultures were centrifuged and washed with liquid fraction three times. Following the final wash, cell pellets were suspended in liquid fraction at the same concentration in which they were washed. Suspensions were added to Mars soil simulant (5 g) in sealed anaerobic tubes at volumes known to result in maximal growth. All three species grew at all three concentrations tested, although some did not grow as well as control cultures did in the standard buffer. This may be a function of the pH. The final pH of the liquid fractions after addition to soil simulant was well below the pH (6.6) of the standard buffer. In a related set of experiments, the three methanogenic species were washed and then inoculated into various dilutions of liquid fraction. *M. wolfei* grew well at all dilutions tested while *M. formicicum* and *M. barkeri* grew well at specific dilutions.

In order to more fully simulate the martian environment, future research plans include attempts to grow the methanogens at varying depths in the Mars soil simulant in a vacuum chamber at the Arkansas-Oklahoma Center for Space and Planetary Sciences.

References: [1] Klein, H.P. (1979) *Rev. Geophys. Space Phys.*, 17, 1655-1662. [2] Allen, C.C. et al. (1998) in *Space 98* (R.G. Galloway and S. Lokaj, eds.) pp. 469-476. American Society of Civil Engineers, NY. [3] Kral, T.A. and Bekkum, C.R. (1999) ISSOL Abstract #P5.23.