

**METHANOGENS: A MODEL FOR LIFE ON MARS.** T.A. Kral<sup>1,2</sup>, T.S. Altheide<sup>2</sup>, A.E. Lueders<sup>1</sup>, T.H. Goodhart<sup>1</sup>, B.T. Virden<sup>1</sup>, W. Birch<sup>1</sup>, K.L. Howe<sup>2</sup> and P. Gavin<sup>2</sup>. <sup>1</sup>Dept. of Biological Sciences, University of Arkansas, Fayetteville, AR, <sup>2</sup>Arkansas Center for Space and Planetary Sciences, University of Arkansas, Fayetteville, AR. tkral@uark.edu.

**Introduction:** The relatively recent discoveries that liquid water most likely existed on the surface of Mars (1) and that methane currently exists in the Martian atmosphere (2, 3, 4) have fueled the possibility of extant or extinct life on Mars. One possible explanation for the methane in the Martian atmosphere would be the presence of methanogens in the subsurface. Methanogens are microorganisms in the domain Archaea that can metabolize H<sub>2</sub> as an energy source, CO<sub>2</sub> as a carbon source, and produce methane.

For the past 16 years, we have been studying methanogens as a model for life on Mars (5, 6, 7, 8, 9). In 1998 (5), we demonstrated that methanogens could exist on relatively low concentrations (down to 15 ppm) of molecular hydrogen, their primary energy source. Theoretically, molecular hydrogen is present, but if not, carbon monoxide has been measured in the Martian atmosphere, and some methanogens can use this in place of molecular hydrogen as an energy source. In 2004 (6), we presented evidence that certain methanogens could grow on a Mars soil simulant (JSC Mars-1). This has been important in trying to simulate the Martian subsurface.

Here we present evidence that certain methanogenic strains can metabolize or survive under a number of conditions present on Mars. These include metabolism at low pressure on JSC Mars-1, metabolism in the presence of perchlorate salts, metabolism using carbonate as a carbon source, and survival following desiccation at Earth-surface pressure (1 bar) as well as Mars-surface pressure (6 mbar).

**Methods:** The methanogens tested were *Methanothermobacter wolfeii*, *Methanosarcina barkeri*, *Methanobacterium formicicum* and *Methanococcus maripaludis*. The low-pressure metabolism experiments were performed in the Andromeda Chamber while the low-pressure desiccation experiments were performed in the Pegasus Chamber. Both vacuum chambers are located at the Arkansas Center for Space and Planetary Sciences, University of Arkansas, Fayetteville. In the low-pressure metabolism experiments, cells were in water-saturated JSC Mars-1. Pressures utilized were 400 mbar and 50 mbar. In the low-pressure desiccation experiments, dried cells were distributed on glass beads (control) or on Martian analog substrates (JSC Mars-1, basalt, montmorillonite, nontronite, jarosite) in open culture tubes. For the perchlorate and carbonate experiments, cultures were grown in standard methanogenic growth media containing the test compounds in anaerobic tubes. The 1 bar desicca-

tion experiments were carried out in desiccators in a Coy Anaerobic Chamber. The dried cells were sitting in open microcentrifuge tubes (1.5 ml) at room temperature in an atmosphere of CO<sub>2</sub> (88%) and H<sub>2</sub> (12%). In all cases, methane was measured by gas chromatography.

**Results and Discussion:** With respect to metabolism at low pressure on JSC Mars-1, significant methane production was observed for *M. wolfeii*, *M. barkeri* and *M. formicicum* at both 400 mbar and 50 mbar (Fig. 1) for up to two weeks (the limiting factor was the availability of liquid water). Even though there are reports of organisms surviving at low pressures, this is one of the first reports of organisms actively metabolizing at these pressures. *M. maripaludis* was not tested in this experiment.

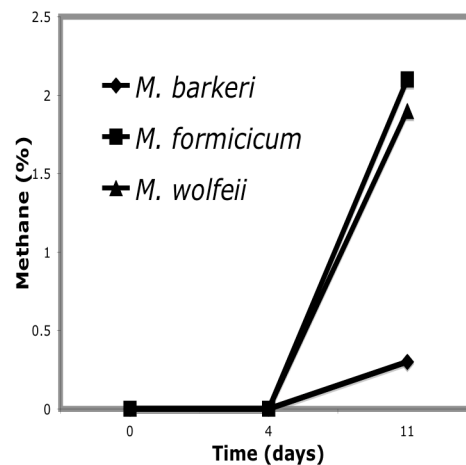


Figure 1. Methane production by *Methanosarcina barkeri*, *Methanobacterium formicicum* and *Methanothermobacter wolfeii* at 50 mbar.

In 2008, The Phoenix Lander detected both perchlorate (10) and carbonate (11) at its landing site. In the presence of perchlorate salts (sodium, magnesium and potassium), all four species of methanogens produced substantial levels of methane, even in the presence of 1% (wt/vol) perchlorate salt. An example is seen in Figure 2.

When CaCO<sub>3</sub> (1% [wt/vol]) was the sole source of carbon, *M. wolfeii*, *M. barkeri* and *M. formicicum* were all able to produce methane, although at much reduced levels compared to cells supplied with CO<sub>2</sub>. An example is seen in Figure 3.

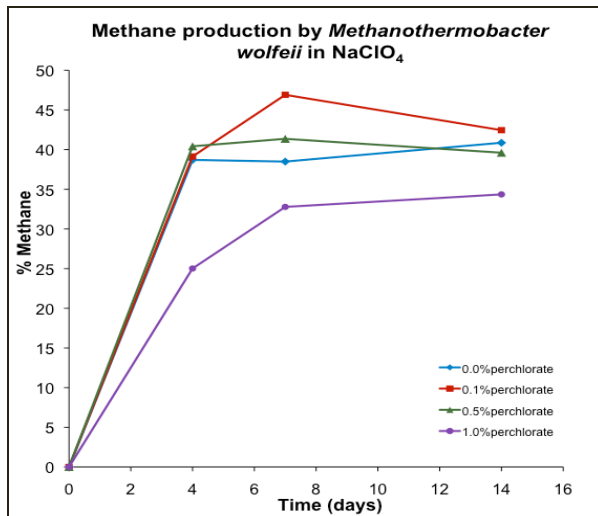


Figure 2. Methane production by *Methanothermobacter wolfeii* in the presence of sodium perchlorate.

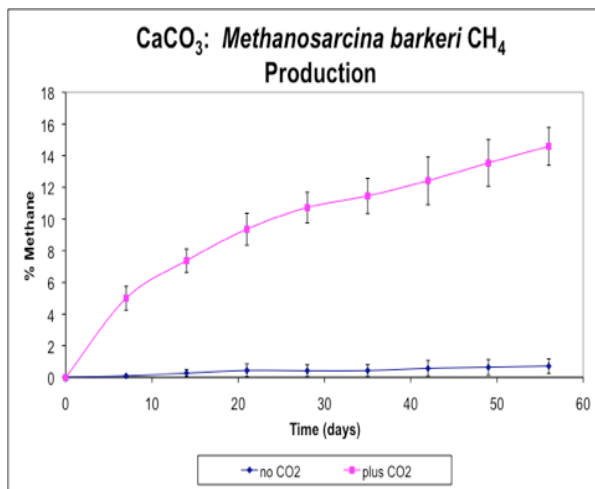


Figure 3. Methane production by *Methanosarcina barkeri* using calcium carbonate or carbon dioxide as carbon source.

In the 1 bar desiccation experiments, *M. barkeri* survived up to 330 days (so far). *M. formicum* survived 180 days while *M. wolfeii* survived 120 days.

In the 6 mbar desiccation experiments, *M. wolfeii*, *M. barkeri* and *M. formicum* survived 120 days (the longest period tested) while *M. maripaludis* survived only 60 days, all on glass. With respect to survival on Martian analog substrates in the desiccated state, *M. barkeri* was the only methanogen tested that survived on multiple substrates (Tables 1 and 2).

	Glass	Substrate*	Glass→Substrate	Substrate→Medium
<i>M. wolfeii</i>	+++	-	+ (SS & Basalt)	+ (Clay & Basalt)
<i>M. barkeri</i>	+++	++ (SS & Clay)	+ (Clay & Basalt)	++ (SS & Clay)
<i>M. formicum</i>	++	-	-	-
<i>M. maripaludis</i>	-	-	-	-

\*JSC Mars-1 (SS)  
Clay (Montmorillonite)  
Basalt

Table 1. Methane production by *Methanothermobacter wolfeii*, *Methanosarcina barkeri*, *Methanobacterium formicum* and *Methanococcus maripaludis* following desiccation at 6 mbar for 90 days.

	Glass Beads	Ch. Basalt	Gr. Basalt	Ch. Jarosite	Gr. Jarosite
<i>M. wolfeii</i>	+++	-	-	-	-
<i>M. barkeri</i>	+++	++	++	++	-
<i>M. formicum</i>	++	-	-	++	-
<i>M. maripaludis</i>	-	-	-	-	-

Ch. = Chunk  
Gr. = Ground

Table 2. Methane production by *Methanothermobacter wolfeii*, *Methanosarcina barkeri*, *Methanobacterium formicum* and *Methanococcus maripaludis* following desiccation at 6 mbar for 120 days.

Overall, the results reported here would seem to indicate that methanogens, as we know them, may be able to survive and possibly thrive in the Martian subsurface.

**References:** [1] Squyres, S.W. et al. (2004) *Science*, 306, 1709-1714. [2] Formisano, V. et al. (2004) *Science*, 306, 1758-1761. [3] Krasnopolsky, V.A. et al. (2004) *Icarus*, 172, 537-547. [4] Mumma, M.J. et al. (2009) *Science*, 323, 1041-1045. [5] Kral, T.A. et al. (1998) *Origins Life Evol. Biosphere*, 28, 311-319. [6] Kral, T.A. et al. (2004) *Origins Life Evol. Biosphere*, 34, 615-626. [7] Kendrick, M.G. and Kral, T.A. (2006) *Astrobiology*, 6, 546-551. [8] Ormond, D.R. and Kral, T.A. (2006) *J. Microbiol. Meth.*, 67, 603-605. [9] McAllister, S.A. and Kral, T.A. (2006) *Astrobiology*, 6, 819-823. [10] Hecht, M.H. et al. (2009) *Science*, 325, 64-67. [11] Boynton, W.V. et al. (2009) *Science*, 325, 61-64.