

ADAPTATION OF METHANOGENS TO INCREASING CONCENTRATIONS OF PERCHLORATE: IMPLICATIONS FOR LIFE ON MARS. T. A. Kral^{1,2}, S. W. McSpadden¹ and G. L. McCracken¹. ¹Dept. of Biological Sciences, University of Arkansas, Fayetteville. ²Arkansas Center for Space and Planetary Sciences, University of Arkansas, Fayetteville (tkral@uark.edu).

Introduction: We have been studying methanogens as a model for life on Mars for 21 years now. The discovery of methane in the martian atmosphere by ground-based and orbital observations as well as Curiosity Rover (1, 2, 3, 4) has added relevance to these types of studies. In 2008, The Mars Phoenix Lander discovered perchlorate at its landing site (5). We have previously discovered that four methanogen species could produce methane in the presence of up to 1% perchlorate wt/vol. The research reported here was designed to determine if methanogens could adapt to higher concentrations of perchlorate.

Methods: The methanogens tested were *Methanothermobacter wolfeii*, *Methanosarcina barkeri*, *Methanobacterium formicicum* and *Methanococcus maripaludis*. Media were prepared containing 1, 2, 5 and 10% magnesium perchlorate wt/vol. Organisms were inoculated into their respective media followed by incubation at each organism's growth temperature. Methane production, commonly used to measure methanogen growth, was measured by gas chromatography of headspace samples. Organisms growing in 1% perchlorate were transferred to tubes containing 2%. Organisms growing in 2% perchlorate were transferred to tubes containing 5%, and so forth.

The standard procedure for growing methanogens typically includes sodium sulfide, a reducing agent, to remove residual molecular oxygen. However, the possibility exists that the sodium sulfide may be reducing the perchlorate, thus allowing for growth of the methanogens. To investigate this possibility, experiments were conducted where stainless steel was used instead of sodium sulfide as

the reducing agent. Prior to inoculation, the stainless steel was removed from the liquid medium.

Results and Discussion: All four methanogens produced substantial methane in the presence of up to 5% perchlorate, but not 10% (Fig.1). Methane concentrations varied with species. When stainless steel was used as a reducing agent, all four methanogens produced methane in the presence of perchlorate. These results indicate that the perchlorates discovered on Mars would not rule out the possible presence of methanogens.

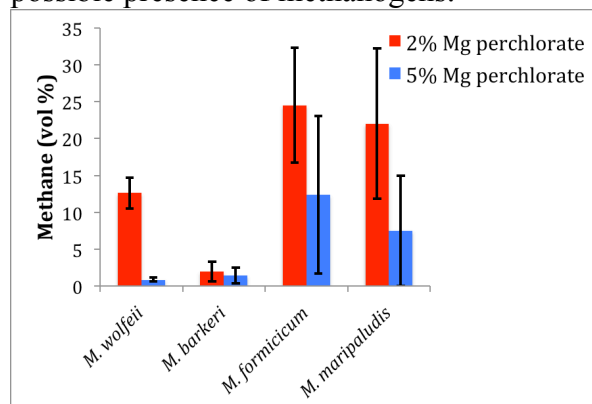


Figure 1. Highest headspace methane concentration following the growth of *Methanothermobacter wolfeii*, *Methanosarcina barkeri*, *Methanobacterium formicicum* and *Methanococcus maripaludis* that were adapted to the presence of 2% and 5% magnesium perchlorate-containing media.

References: [1] Formisano, V. et al. (2004) *Science*, 306, 1758-1761. [2] Krasnopolsky, V.A. et al. (2004) *Icarus*, 172, 537-547. [3] Mumma, M.J. et al. (2009) *Science*, 323, 1041-1045. [4] Webster, C.R. et al. (2014) *Scienceexpress*, DOI:10.1126/science.1261713. [5] Hecht, M.H. et al. (2009) *Science*, 325, 64-67.