METHANE PRODUCTION BY METHANOGENS IN THE ANDROMEDA ENVIRONMENTAL CHAMBER UNDER CONDITIONS APPROACHING THOSE OF THE MARTIAN ENVIRONMENT. T. A. Kral, D. W. G. Sears, P. H. Benoit, and M. S. Kareev, Arkansas-Oklahoma Center for Space and Planetary Sciences, University of Arkansas, Fayetteville, AR 72701.

Introduction: Surface conditions on Mars are probably too hostile for life, as we know it, to exist [1, 2, 3]. Below the surface, however, is another matter. If subsurface liquid water is present [4, 5], then conditions may be conducive to the growth of methanogens. Methanogens, members of the domain Archaea, are microorganisms that are found deep below the surface of the Earth as well as deep within the oceans [6, 7, 8].

Materials and Methods: In an attempt to determine if species of methanogens can grow (or just survive) under conditions approaching those at the surface of Mars, we inoculated JSC Mars-1, a Mars soil simulant [9] with *Methanobacterium formicicum, Methanosarcina barkeri*, and *Methanothermobacter wolfeii* in the Andromeda Environmental Chamber at the Arkansas-Oklahoma Center for Space and Planetary Sciences. The Andromeda Chamber is a 4 m tall by 1 m wide vacuum cylinder that has been designed to simulate planetary surface conditions. Cultures were grown in their respective media [10, 11], washed and suspended in a standard carbonate buffer, and frozen in anaerobic culture bottles. The frozen cultures were placed inverted into the soil simulant that was in a stainless steel bucket. The bucket was lowered into the chamber, the chamber sealed, and the atmosphere replaced with equal volumes of carbon dioxide and molecular hydrogen at a pressure of 400 mbar. The atmosphere was replaced before the cultures melted in order to prevent the methanogens from coming in contact with atmospheric oxygen (methanogens are strict anaerobes). Palladium catalyst boxes were mounted on the top of the bucket to help remove residual oxygen. In some experiments, anaerobic 4.0 mM sodium sulfite solution was poured into the soil as an additional measure to remove residual oxygen [12].

Results and Conclusions: All three species survived a week under the described conditions, *M. wolfeii* showing the greatest increase and *M. formicicum* showing the smallest increase in methane production. Sodium sulfite additions resulted in even greater methane production. These experiments demonstrated methane production at reduced pressure, survival of the organisms after a freeze/thaw cycle, and the usefulness of the Andromeda Chamber.

References: [1] Klein H. P. (1978) *Icarus*, 34, 666-674. [2] Klein H. P. (1979) *Rev. Geophys. Space Phys*., 17, 1655-1662. [3] Klein H. P. et al. (1992) *Mars*, 1221-1233. [4] Boynton W. V. et al. (2002) *Science*, Abstract #1073722v1. [5] McKay C. P. and Stoker C. R. (1989) *Rev. Geophys*., 27, 189-214. [6] DiMarco A. A. et al. (1990) *Ann. Rev. Biochem*., 59, 355-394. [7]Jarrell K. F. and Kalmokoff M. L. (1987) *Can. J. Microbiol*., 34, 557-576. [8] Jones W. J. et al. (1983) *Arch. Microbiol*., 136, 254-261. [9] Allen C.C. et al. (1998) *Space 98*, 469-476. [10] Boone D. R. et al. (1989) *Appl. Environ. Microbiol*., 55, 1735-1741. [11] Xun L. et al. (1988) *Appl. Environ. Microbiol*., 54, 2064-2068. [12] Rothe O. and Thomm M. (2000) *Extremophiles*, 4, 247-252.