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Growth of Methanogens on a Mars Soil Simulant Under Simulated Martian Conditions

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Abstract. Due to the hostile conditions at the surface, any life forms existing on Mars today would most likely inhabit a subsurface environment where conditions are potentially wetter and warmer, but organic compounds may be lacking and light energy for photosynthesis would be absent. Methanogens, members of the domain Archaea, are microorganisms from planet Earth that can grow under these relatively extreme conditions. We have demonstrated that certain methanogenic species can indeed grow on a Mars soil simulant, JSC Mars-1, with limited amounts of water, under conditions approaching a possible subsurface environment on Mars.

1. Introduction

The Viking Landers only examined the surface of Mars where conditions are probably too dry, too cold, and too oxidizing for known life forms to exist (Klein 1978; Klein 1979). It may be possible that liquid water can exist and persist below the surface (Boynton et al. 2002; Feldman et al. 2002; Mitrofanov et al. 2002; McKay & Stoker 1989; McKay et al. 1992; McKay 1997). Life forms existing below the surface could not obtain their energy from photosynthesis, but rather they would have to utilize chemical energy. Because the Viking Landers found no measurable quantities of organic matter, life forms might be limited to oxidation of inorganic matter for energy. Organisms that fall into this category are referred to as chemoautotrophs. Methanogens are chemoautotrophs that consume molecular hydrogen and carbon dioxide and produce methane as a waste product. A potential habitat for existence of methanogens on Mars might be a geothermal source of hydrogen, possibly due to volcanic or hydrothermal activity, or the reaction of basalt and anaerobic water (Boston et al. 1992; Stevens & McKinley, 1995), carbon dioxide, which is abundant in the martian atmo-

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sphere, and subsurface liquid water. The reaction could conceivably proceed in the dark at depth in the martian soil.

The research reported here demonstrates that certain methanogens can grow on a Mars soil simulant when supplied with carbon dioxide, molecular hydrogen, and limiting amounts of water.

2. Matherials and Methods

2.1. Cultures and Growth Media

The methanogens were obtained from David Boone, Oregon Graduate Institute, Beaverton, OR (now at Portland State University). Each methanogenic strain was grown in a standard medium that supported optimal growth (MS medium, Boone et al. 1989) for *Methanosarcina barkeri* and *Methanobacterium formicicum*; MM medium (Xun et al. 1988) for *Methanothermobacter wolfeii* (formerly *Methanobacterium wolfei*); and MSH medium (Ni & Boone 1991) for *Methanococcus maripaludis*). Growth media were prepared under 95% carbon dioxide and 5% hydrogen in a Coy environmental chamber. Methanogens are strict anaerobes and will not grow or produce methane in the presence of molecular oxygen (Zinder 1993). The anaerobically-prepared media were added to growth vessels in the anaerobic chamber. Each growth vessel was sealed with a butyl rubber stopper. Outside of the chamber, each growth vessel was crimped with an aluminum cap, and then molecular hydrogen was added to each vessel using a gassing manifold. At least one hour prior to inoculation, a sterile sodium sulfide solution was added to each vessel to eliminate any residual molecular oxygen (Boone et al. 1989).

2.2. Growth on Mars Soil Simulant

Actively growing cells (approximately 0.1 O.D. at 675 nm) were centrifuged at 4200 rpm for 45 min, and then washed with sterile carbonate buffer (the same buffer used to make methanogenic growth medium). At least an hour earlier, sterile sodium sulfide solution was added to the buffer to eliminate residual oxygen. This washing procedure was repeated three times. Following the final washing, the cell pellets were suspended in the same buffer. Various volumes of each cell suspension were added to individual tubes containing 5 g of sterile Mars soil simulant, or serum bottles containing 10 g of the simulant. Dr. Carlton Allen of Lockheed Martin Space Mission Systems & Services, Houston, TX, has provided Mars soil simulant, JSC Mars-1, derived from altered volcanic ash from a Hawiian cinder cone (Allen et al. 1998). This soil simulant approximates the composition, grain size, density, and magnetic properties of martian soil. Prior to each experiment, JSC Mars-1 samples were placed into anaerobic tubes or bottles. The unstoppered tubes and bottles were placed into the anaerobic chamber and allowed to sit overnight to allow for removal of residual molecular oxygen. They were then stoppered in the chamber, removed, crimped, and then sterilized in an autoclave. As a positive control, 0.5 mL of the cell suspension was added to standard growth medium to make sure that the cells were not killed in the washing procedure. One negative control consisted of the cell suspension only. (Cells should not grow for a significant amount of time in buffer alone.)

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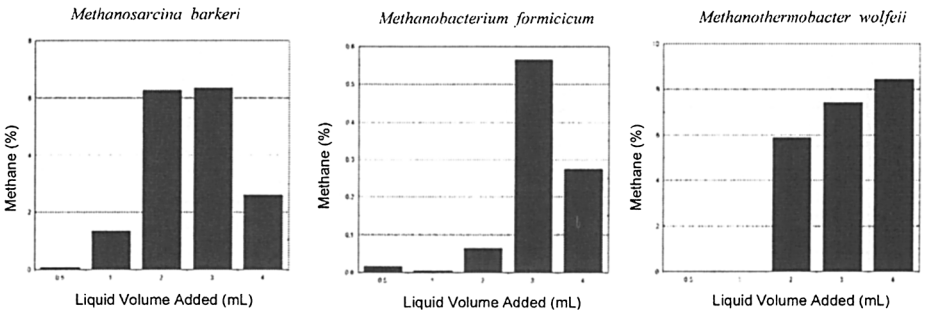

Figure 1. Methane production by *Methanosarcina barkeri, Methanobacterium formicicum* and *Methanothermobacter wolfeii* on JSC Mars-1 soil simulant in 25 mL anaerobic tubes with varying amounts of water (buffer) following two weeks of incubation.

Another negative control consisted of sterile buffer being added to Mars soil simulant. A final negative control consisted of the Mars soil simulant without water or microorganisms. All tubes and bottles were pressurized with 200 kPa (above ambient) of 75:25 hydrogen:carbon dioxide. The tubes and bottles were incubated at temperatures within the growth range for the respective methanogens (37°C for *M. barkeri* and *M. formicicum*; 25°C for *M. maripaludis*, and 55°C for *M. wolfeii*). Growth was measured by methane production. Headspace gas samples (1 mL) were removed at time intervals and analyzed by a Hewlett Packard model 5890 gas chromatograph with a thermal conductivity detector at an oven temperature of 40°C using argon as the carrier gas.

3. Results

Of the four species of methanogens tested, *M. wolfeiii*, *M. barkeri* and *M. formicicum* were able to produce methane when on Mars soil simulant with reduced amounts of water (Figs. 1 and 2). So far, *M. maripaludis* has shown no methane production on Mars soil simulant. Figure 1 shows methane production of the methanogens in 25 mL culture tubes containing 5 g of Mars soil simulant after two weeks of incubation. *M. wolfeii* showed methane production when 2 mL or more of cell suspension were added to 5 g of Mars soil simulant. As the water content increased up to standing liquid and greater, the methane increased. (Volumes greater than 3.5 mL resulted in standing liquid.) *M. barkeri* produced methane fairly well when 1 mL was added to the 5 g of soil simulant. In 25 mL anaerobic culture tubes, *M. barkeri* always demonstrated greater methane production when standing liquid was not present. *M. formicicum* appears to show results similar to *M. barkeri*, producing more methane with less water. However, results in Figure 1 are after 2 weeks of incubation. With longer incubation periods, the methane production pattern for *M. formicicum* resembles that for *M. wolfeii*, with increasing water leading to greater methane production. Figure 2 shows methane production by the same methanogens in 150 mL serum bottles as a function of time. *M. barkeri* behaved more like *M. wolfeii*, showing increasing

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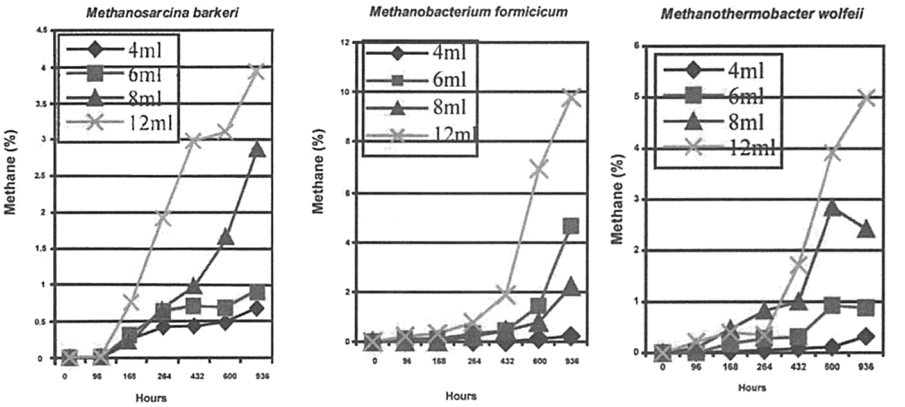

Figure 2. Methane production by *Methanosarcina barkeri, Methanobacterium formicicum* and *Methanothermobacter wolfeii* on JSC Mars-1 soil simulant in 150 mL anaerobic bottles with varying amounts of water (buffer) as a function of time.

methane production with increasing water. This difference in responses seen for *M. barkeri* in tubes vs. bottles may be related to surface area interactions between the soil simulant and the gaseous phase. The control experiments behaved as expected.

4. Discussion

Results show that three of four strains of methanogens tested were able to produce methane when inoculated into a Mars soil simulant with varying amounts of water. The *M. wolfeii* and *M. formicicum* species both showed increasing methane production in response to increasing water. *M. barkeri*, on the other hand, produced more methane when less water was present in 25 mL tubes, but mimicked the other two species in 150 mL bottles. The lack of growth of *M. maripaludis* in our experiments may be associated with its halophilic nature. It requires a higher salt concentration for growth, compared to the other three methanogens tested.

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