

Growth of Methanogens in the Presence of Perchlorates: Implications for Life on Mars.
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We have been studying methanogens as a model for life on Mars for 19 years now. The discovery of methane in the Martian atmosphere has added relevance to these types of studies. In 2008, The Mars Phoenix Lander discovered perchlorate at its landing site. In 2012, the Curiosity Rover has confirmed the presence of perchlorate. Because of perchlorate's powerful oxidizing property, many believed that the chances for extant life on the planet had decreased. The research reported here was designed to determine if methanogens could grow in the presence of three different perchlorate salts. The methanogens tested were *Methanothermobacter wolfeii*, *Methanosarcina barkeri*, *Methanobacterium formicicum* and *Methanococcus maripaludis*. Media were prepared containing 0, 0.1, 0.5 and 1.0% magnesium perchlorate, sodium perchlorate, or potassium perchlorate. Organisms were inoculated into their respective media followed by incubation at each organism's optimal growth temperature. Methane production, commonly used to measure methanogen growth, was measured by gas chromatography of headspace samples. Methane concentrations varied with species and perchlorate salt tested. However, all four methanogens produced substantial levels of methane, even in the presence of 1.0% perchlorate. In all cases, there was no difference in methane concentrations at 0 and 0.1% perchlorate. In most cases, 1.0% perchlorate resulted in lesser amounts of methane, at least initially. The standard procedure for growing methanogens typically includes sodium sulfide, a reducing agent, to remove residual molecular oxygen. (Methanogens are strict anaerobes.) 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 zerovalent iron was used instead of sodium sulfide as the reducing agent. Prior to inoculation, the iron was removed from the liquid medium. Just as in the prior experiments, the methanogens demonstrated growth in the presence of perchlorate. Currently, experiments are in progress to determine if these methanogens can adapt to higher concentrations of perchlorate. It appears that some can grow in the presence of 5% perchlorate. The results reported here indicate that the perchlorates discovered by the Phoenix Lander and the Curiosity Rover would not rule out the presence of methanogens on Mars.