METHANE PRODUCTION BY METHANOGENS IN PERCHLORATE-SUPPLEMENTED MEDIA. Howe, K. L.[1], Gavin, P.[1], Goodhart, T.[2] and Kral, T. A.[1, 2], [1]Arkansas Center for Space and Planetary Science, University of Arkansas, Fayetteville, AR 72703, [2]Department of Biological Sciences, University of Arkansas, Fayetteville, AR 72703

Introduction: Perchlorate (ClO_4^-), a highly oxidizing compound, was identified on the martian surface by the Phoenix Lander. Concentration levels as high as 1.0% perchlorate have been detected by the microscopy, electrochemistry and conductivity analyzer (MECA) on board the lander. The thermal and evolved gas analyzer (TEGA) was unable to confirm the presence of perchlorates. When this compound was originally discovered by Phoenix's science instruments, enthusiasm about the possibility of life was dampened, as some scientists think perchlorates too toxic and oxidizing a substance to support life. There is some debate as to whether an environment with perchlorate salts would be too harsh for any organism to survive.

Methanogens are anaerobic chemoautotrophs that consume carbon and produce methane using an energy source such as molecular hydrogen [1]. They are microorganisms that are able to survive in a plethora of unwelcoming environments. Due to their ability to survive in extreme conditions, methanogens may have been the first autotroph to evolve on Earth [1]. It is not impossible to imagine, then, that methanogens may have evolved throughout Mars's history.

Methods: In order to test for possible methanogen growth in a perchlorate salt medium, four methanogen species were chosen due to their previous success of surviving in Mars-like conditions [1]. An appropriate medium was prepared for each methanogen being tested: MM for *Methanothermobacter wolfeii*; MS for *Methanosarcina barkeri*; MSF for *Methanobacterium formicicum*; and MSH for *Methanococcus maripaludis*. Carbon dioxide was bubbled through the medium to provide a carbon source for the methanogens. The beakers of media were placed uncovered in a carbon dioxide chamber overnight. A perchlorate concentrated stock solution was also made and placed in the chamber.

In the chamber, sixteen tubes were each filled with 5 ml of the appropriate medium. Each tube was sealed with a butyl rubber stopper and removed from the chamber. After crimping, the tubes were sterilized in an autoclave. Different concentrations of sterile perchlorate salt solutions (K, Na, and Mg) were added to the media tubes resulting in final perchlorate concentrations of 0, 0.1, 0.5 and 1.0%. Twelve drops of sterile sodium sulfide (2.5%) were added to each tube to eliminate any residual oxygen. Tubes were inoculated with the appropriate organism, then pressurized with a hydrogen energy source. Incubation occurred at ideal temperature for each species tested. Methane production was measured by gas chromatography.

Results: All four species of methanogens produced detectable amounts of methane at all concentrations of each salt tested.

Potaasium perchlorate. Methane produced by *M. wolfeii* reached peak production within the first week of incubation and leveled off to a fairly consistent level for the rest of the experiment. Perchlorate concentration had very little effect on methane levels. *M. formicicum* showed similar results with a slight decrease in initial methane concentration at 1.0% perchlorate. *M. barkeri* had a very linear relationship between time and methane with all perchlorate concentrations during the duration of the experiment. Throughout the experiment there was less methane measured at 1.0% perchlorate. *M. maripaludis* appeared to be the most effected by higher perchlorate concentration, especially 1.0% (Figure 1).

Sodium perchlorate. Again, *M. wolfeii* and *M. formicicum* showed similar methane patterns with slightly less methane at 1.0% sodium perchlorate. *M. barkeri* again demonstrated linear patterns of methane concentration, however, 0.5 and 1.0% perchlorate resulted in much lower methane levels than those seen at 0 and 0.1%. *M. maripaludis* showed similar methane levels at 0, 0.1 and 0.5% perchlorate, but considerably less at 1.0% (Figure 1).

Magnesium perchlorate. Similar patterns were observed for *M. wolfeii, M. formicicum* and *M. maripaludis* (Figure 1) grown in the presence of magnesium perchlorate. All showed decreased methane concentrations initially in the presence of 1.0% perchlorate with lesser effects at 0.5%. *M. barkeri*, on the other hand, demonstrated less of an effect of 0.5 and 1.0% magnesium perchlorate compared to both potassium and sodium perchlorate experiments.

Discussion: 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

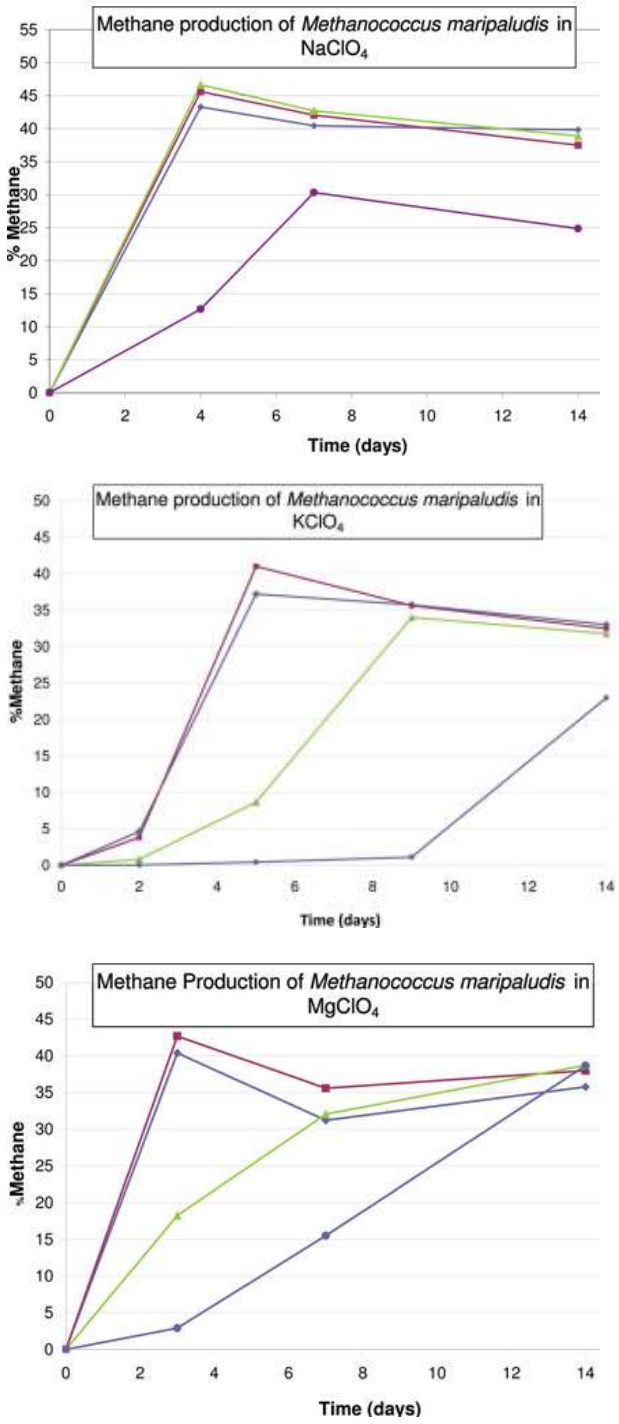

Figure 1. Methane concentrations in *Methanococcus maripaludis* cultures exposed to three perchlorate salts. Diamonds represent the control, squares the 0.1% perchlorate, triangles the 0.5% perchlorate and the circles 1.0% perchlorate.

least initially. There are at least two possible explanations for this. The higher perchlorate concentrations may be inhibiting methane production by the methanogens. A second explanation would be that methanogenesis is not being inhibited, but the methane being produced is being oxidized by the perchlorate, resulting in less methane being measured in the headspace of the culture tubes. In some cases (*M. wolfeii* and *M. formicicum*), methane concentrations in the higher-concentration perchlorate tubes eventually reach the level of the control, which would support the second explanation. The perchlorate concentration may be decreasing as it is used up oxidizing the methane. With respect to *M. barkeri* and *M. maripaludis*, it may be a matter of time before the methane eventually reaches control levels. There may be some inhibition of methanogenesis, or a combination of both explanations. Nonetheless, substantial methane was produced by all four methanogens at all perchlorate concentrations tested.

Implications for Mars: Although globally methane in the martian CO_2 atmosphere is dilute, about 10 ppb, there are localized areas where the concentrations are as high as 35 ppb and must be constantly replenished due to photochemical losses [2]. These localized areas and concentrations cannot be explained by impacts or volcanism [2] and may be areas where methanogens are producing methane. Results here indicate that the perchlorates discovered by the Phoenix lander would not necessarily rule out the presence of these methanogens on Mars.

References: [1] Kral, T. A., Bekkum, C. R., and McKay, C. P., (2004) *Origins of Life and Evolution of the Biosphere*, *34*, 615-626. [2] Barlow, N. (2008), Mars: Cambridge, Cambridge University Press.