PERCHLORATE REDUCING BACTERIA: EVALUATING THE POTENTIAL FOR GROWTH UTILIZING NUTRIENT SOURCES IDENTIFIED ON MARS. K. F. Bywaters[1] and R. C. Quinn[2], [1]NASA Ames Research Center, Moffett Field, Mountain View, CA 94035 (kathryn.f.bywaters@nasa.gov), [2]SETI Institute/NASA Ames Research Center (richard.c.quinn@nasa.gov).

Introduction: Perchlorate reducing bacteria may serve as model organisms for potential life forms that could exist on Mars. Due to the high reduction potential of perchlorate it can be utilized in microbial metabolism as an electron acceptor [1]. Perchlorate was discovered to be present in martain surface materials (~0.5% wt.) using the Wet Chemistry Laboratory and the Thermal and Evolved Gas Analyzer on the Phoenix Lander [2]. An apparent widespread distribution of perchlorate on Mars was confirmed using the Sample Analysis at Mars (SAM) instrument suite on the Mars Curiosity Rover [3].

Nutrient sources that may support microbial metabolism on Mars potentially exist in the form of carbon as formate, nitrogen as nitrate, and phosphorus potentially as phosphate. Formic acid synthesis may occur on Mars due to UV irradiation of CO, CO_2 and small quantities of water vapor [4]. The SAM investigation has detected oxidized nitrogen-bearing compounds on Mars and these results support nitrate in concentrations ranging from 110-1100 ppm [5]. The analysis of elemental composition of the "Paso Robles" evaporitic deposits on Husband Hill, using the Athena Science instrument package on Spirit, indicates the presence of Ca-phosphate [6]. To evaluate the potential for microbial growth through the utilization of these nutrients, perchlorate-reducing bacteria were cultured in media containing phosphate and different combinations of nitrogen and carbon sources, including formate and nitrate.

Methods: *Azospira suillum* strain PS (formally *Dechlorosoma suillum* strain PS) was anaerobically grown in 4 different media treatments: 1) 5 mM ammonium chloride, 10 mM sodium acetate, 5 mM magnesium perchlorate, 5 mM monosodium phosphate, 7 mM disodium phosphate; 2) 8 mM sodium nitrate, 10 mM sodium acetate, 5 mM magnesium perchlorate, 5 mM monosodium phosphate, 7 mM disodium phosphate; 3) 10 mM sodium nitrate, 20 mM sodium formate, 5 mM monosodium phosphate, 7 mM disodium phosphate, 5 mM magnesium perchlorate; 4) 10 mM sodium nitrate, 10 mM sodium formate, 5 mM monosodium phosphate, 7 mM disodium phosphate, 5 mM magnesium perchlorate. Five replicates were done for all treatments.

Growth curves of *A. suillum* strain PS for each treatment were obtained by measuring optical density at 600 nm using an Ocean Optics (HR4000CG-UV-NIR) spectrometer and a halogen (HL-2000, Ocean Optics, Inc.) light source. To obtain growth curves, measurements were taken approximately every 24 hours over a 5-day period.

Growth rates were determined using:

$$\mu = [\ln(A_{600;\,t2}) - \ln(A_{600;\,t1})]/\Delta t$$

where μ is the growth rate, $\ln(A_{600;\,t2})$ is the natural log of the absorbance reading at the end of the determined growth phase, $\ln(A_{600;\,t1})$ is the natural log of the absorbance reading at the beginning of the determined growth phase and Δt is the time interval.

Results: Figure 1 shows the growth curves for the four different treatments. Growth rates during the exponential growth phase (between time steps 72 and 84 hours) for the treatments are shown in Figure 2. Growth rates between treatment 1 and 2 showed no significant difference. The final measured biomass values for treatment 1 or 2 were 20% higher when compared with treatment 3.

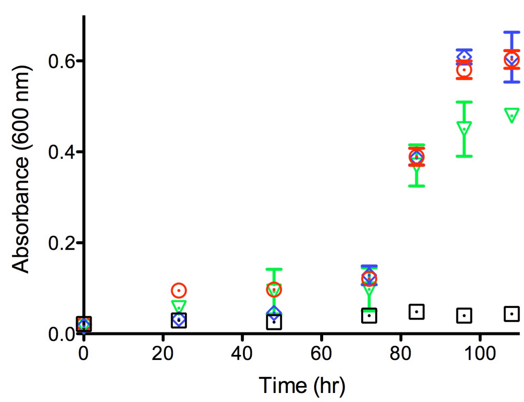

Figure 1. Growth curves for *A. suillum* strain PS cultured with media containing phosphate and different combinations of nitrogen and carbon sources. The four different media treatments were: 1) 5 mM ammonium chloride and 10 mM sodium acetate (blue); 2) 8 mM sodium nitrate and 10 mM sodium acetate (red); 3) 8 mM sodium nitrate and 20 mM sodium formate (green); 4) 8 mM sodium nitrate and 10 mM sodium formate (black).

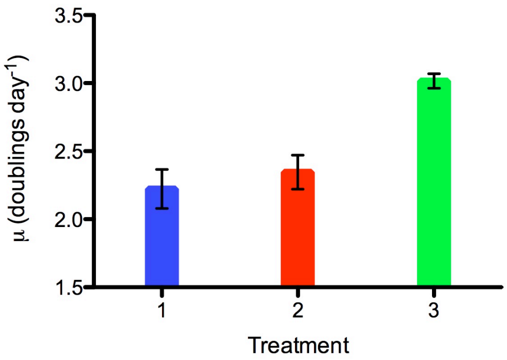

Figure 2. Growth rates during the exponential growth phase (between time steps 72 and 84 hours, fig. 1) for *A. suillum* strain PS cultured with media containing phosphate and different combinations of nitrogen and carbon sources. Three different media treatments were: 1) 5 mM ammonium chloride and 10 mM sodium acetate (blue); 2) 8 mM sodium nitrate and 10 mM sodium acetate (red); 3) 8 mM sodium nitrate and 20 mM sodium formate (green).

Summary: The discovery of the high levels of perchlorate on Mars indicates the presence of an energy source that may be available for bacterial utilization. Our results show that perchlorate-reducing bacteria, which may provide a good analogy for potential life on Mars, are capable of utilizing the nutrients available in martian surface materials.

Our experiments show that the perchlorate-reducing bacteria *A. suillum,* grows in treatment 3 media which contains 20 mM formate and 10 mM nitrate. However, *A. suillum,* does not grows in treatment 4 media containing 10 mM formate and 10 mM nitrate. We have demonstrated that *A. suillum* can utilize formate in combination with nitrate. Previous findings have shown that *A. suillum* strain PS does not grow in media containing 10 mM formate and 5 mM ammonium, but *A. suillum* strain MissR does [8]. Given the results of Stern et al. [5], nitrate may be a more viable nitrogen source on Mars compared to ammonium. Although Phoenix Wet Chemistry Laboratory data may show possible low levels of ammonium [9], it is unclear if these levels are significant relative to measurement error, sensor cross sensitivities, and potential contamination. The final measured biomass values for all treatments in our experiments indicate that although sodium acetate may be more efficiently unitized, sodium formate is a viable electron donor. These results will be discussed in the context of on going growth experiments, which examine water availability of hydrated perchlorate salts and brines.

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