Investigating the Coexistence of Perchlorate Reducing Bacteria and Naturally Occurring Perchlorate-Rich Sediments in the Pilot Valley Paleolake Basin.
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Introduction: The presence of perchlorate salts on Mars suggests a possible energy resource for microorganisms. Perchlorate Reducing Microorganisms (PRM) are seemingly ubiquitous on Earth as they have been isolated from a variety of environments including gold mine drainage sites, contaminated and pristine groundwater sites, wastewater sludge, natural swamps, and from deep sea sediments. PRM are also phylogenetically diverse, occurring in both the Bacterial and Archaeal domains [3-5]. Surprisingly, little evidence exists documenting the existence of PRM in settings where naturally occurring ClO_4^- is abundant [6]. Hence, finding these two entities together in a relevant analog environment on Earth would make for an excellent model for this kind of ecosystem on Mars. Furthermore, this finding could help to shed light on the evolutionary history of microbial perchlorate reduction.

Here, we present evidence that PRM co-exist with naturally occurring perchlorate in the Pilot Valley basin of the Great Salt Lake Desert, and offer this as a potential model for the study of microbial metabolisms that could occur in ancient paleolake basins on Mars.

Field Site and Sample Collection: Pilot Valley is a closed basin paleolake that contains perchlorate-rich sediments. The four sediment samples (SLD113, SLD114, SLD115 and SLD124) used for experiments in this study were taken from putative MISS structures discovered at site PV-4 of Pilot Valley study transect [9]. Upon collection, samples were placed in vacuum-sealed bags and stored at 4°C prior to incubation in medium designed to enrich for PRM in a near-native environment.

Methods: *Enrichment Cultures.* Two sets of microcosm experiments were established at 0.1% and 100-ppm final perchlorate concentrations. The microcosms were allowed to incubate for 371 days and 285 days respectively along with inoculum free blanks. Samples removed from the microcosms were stored at -80°C prior to analysis.

DNA Extraction and Sequencing. Bulk DNA was extracted using the MoBio PowerSoil kit. DNA from the 6 most concentrated extractions of the 0.1% experiment were prepped and 16S-sequenced on the Illumina MySeq platform. The resulting sequences were processed in Qiime 1.8 [10].

qPCR Analysis. Quantitative PCR analysis of the chlorite dismutase (*cld*) gene and the perchlorate reductase (*pcrA*) gene were conducted using DNA from the same 6 high concentration samples.

Perchlorate and Chloride Analysis. Perchlorate (ClO_4^-) was measured on all microcosms in both experiments by sequential ion chromatography-mass spectroscopy/mass spectroscopy (IC-MS/MS). Chloride concentrations were measured using Ion Chromatography to rule out sample contamination.

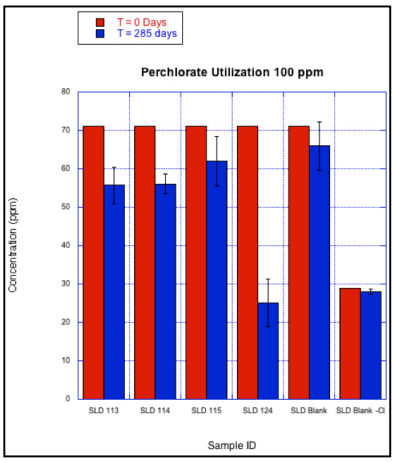

Figure 1. Results from 100-ppm perchlorate utilization experiment

Summary: Results from both utilization experiments show statistically significant reductions in perchlorate across all microcosms. Analysis of the blank controls shows an insignificant reduction in perchlorate concentration and an insignificant increase in chloride concentration (an indicator of contamination). qPCR results show copy numbers of the *cld* gene that correspond to a PRM abundance equal to 1% of the total microcosm population. 16S sequencing results also show that there is a small population of known PRM present in the microcosms at approximately 1% (in correlation with qPCR results). These results suggest that PRM co-exist in the Pilot Valley basin with the naturally occurring perchlorate present in the sediments. These results could explain the perchlorate concentration gradient seen across the Pilot Valley study transect, though further study is necessary to verify that PRM are actively utilizing the perchlorate in the basin sediments.

References: [1] Liebensteiner et al. (2013) *Sci*., 340,85-87. [2] Nerenberg, R. (2013) *Sci*., 340, 38-39. [3] Oren et al. (2014) *Extremo.*,18, 75-80. [4] Liebensteiner et al. (2015) *Ann. N.Y. Acad. Sci.* ISSN 0077-8923. [7] Lynch et al. (2015) *JGR Planets*, 120, 599-623.