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Curtis-Harper, E.; Pearson, V. K.; Schwenzer, S. P. and Olsson-Francis, K. (2014). The anaerobic community of an estuarine environment: an analogue for life on Mars. In: 12th Early Career Planetary Scientists' Meeting, 19-20 Feb 2015, University of Kent, UK Planetary Forum.

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The anaerobic community of an estuarine environment: an analogue for life on Mars.

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Introduction:

The first step in finding potential extant, and/or extinct, life on Mars is to understand the potential biological processes that may have occurred on Mars and identify biosignatures that such processes would generate. This is dependent on identifying and characterising microbial life in suitable terrestrial analogue environments and reliably distinguishing between biotic and abiotic processes. Chemolithotrophic anaerobic microorganisms, such as methanogens, are ideal organisms for investigating potential life in the martian sub-surface^[1] as they represent deeply branched terrestrial species that would likely survive there. Furthermore, the carbon dioxide and hydrogen required for their metabolism are provided by the approximately 96% carbon dioxide atmosphere^[2] and hydrogen produced in serpentinisation and other reactions^[3].

In this study, we used a community of anaerobic microorganisms (Fig.1), which were isolated from below the redox potential discontinuity (RPD) layer of the River Dee estuary, UK. The anaerobic conditions, the 11-15°C temperature and high salinity (37g/l NaCl) make the sub-RPD zone an ideal analogue for the martian subsurface.

Methods:

Using MiSeq sequencing we investigated the composition of the microbial community, which included sulfate reducing bacteria (Fig. 1). Anaerobic growth experiments were conducted using a minimal growth medium containing dd H₂O, sodium lactate (carbon source), ammonium chloride (nitrogen source) and thioglycollate and ascorbic acid reducing agents. The remaining bioessential elements were provided by a Mars analogue rock mix consisting of a non-amygdaloidal terrestrial basalt from Le Cheix Quarry and aegirine in the ratio 1.75:1 respectively. These geological substrates were geochemically characterized by XRF and XRD and shown to be a compositional analogue for the Rocknest site on Mars^[4].

Growth was measured using cell counts. The dissolution kinetics will be determined by analysing the release of key elements, such as Si, Ca, K, Fe in the growth medium with ICP-AES (results pending). Gas Chromatography was carried out on aliquots of the headspace gas to investigate the presence of methane, which is a byproduct of methanogen metabolism.

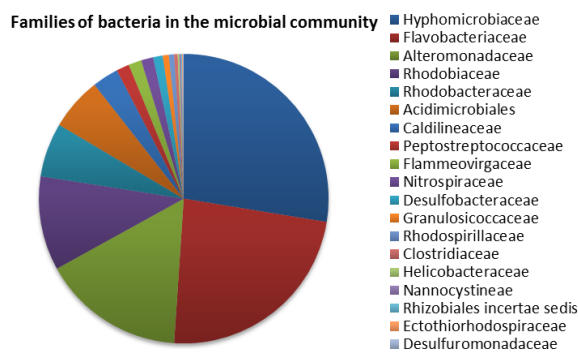
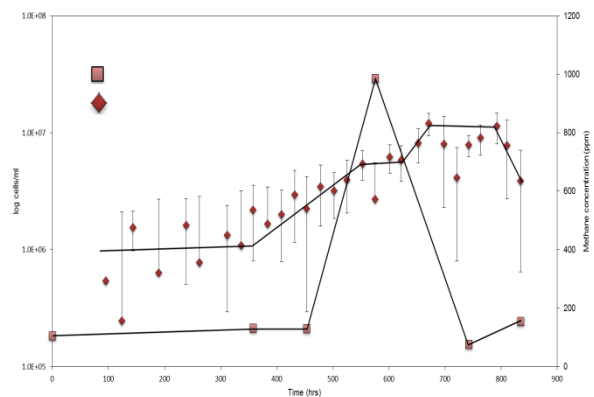


Figure 1. MiSeq analysis on DNA extracted from the River Dee sub-RPD sample.



Results/Discussion:

The growth curve displayed in Fig. 2 demonstrates that the microbial community was able to grow in the nutrient poor minimal growth medium, utilising the bio-essential elements provided by the Mars analog substrates. Abiotic controls were treated identically and no growth was observed. Thus a varied anaerobic microbial community, which contains chemolithotrophs, can utilize a Mars analogue rock substrate.

The growth curve suggests a possible biphasic growth. The simultaneous fluctuations in methane concentrations may suggest a relationship between methanogenic and methanotrophic microorganisms whereby the secondary exponential growth phase can be attributed to methanotrophs utilizing methane produced when the methanogenic archaea exponentially grow. MiSeq sequencing has already been used to identify the presence of methanotrophic bacteria in our samples and further analysis of the archaeal community, using MiSeq analysis, will confirm whether methanogenic archaea are present.

Further experimental work will focus on identifying geochemical biomarkers formed as a result of the presence of the microbial community by comparing ICP-AES data for media aliquots taken from biotic and abiotic samples.

Conclusions:

This study demonstrates that the microbial community below the RPD of the River Dee Estuary can act as an informative analogue in studies of Martian habitability and life detection. Thus it should be possible to identify biosignatures in the Mars analogue substrate to inform future Mars life detection missions.

References: [1] Weiss B. P., Yung Y. L. and Neelson K. H. 2000. *PNAS*. 97(4):1395–1399. [2] Mahaffy P. et al 2013 *Science*, 341(1997): 263-266 [3] Hellevang H., Huang S. and Thorseth I. 2011 *Astrobiology* 11(7): 711-724. [4] Schmidt M., Campbell J. and Gellert R. 2014 *Journal of Geophysical Research: Planets* 119(1): 64-81