High-throughput sequencing reveals diverse microbial communities in Icelandic Mars Analog Environments

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Introduction: Exploration missions to Mars rely on landers or rovers to perform multiple analyses over geographically small sampling regions while landing site selection is done using large-scale but low-resolution remote sensing data. Utilizing Earth analog environments to estimate small-scale spatial and temporal variation in key geochemical signatures and biosignatures will help mission designers ensure future sampling strategies will meet mission science goals. Icelandic lava fields can serve as Mars analog sites due to conditions that include low nutrient availability, temperature extremes, desiccation, and isolation from anthropogenic contamination. Previous work at Icelandic Mars analog sites found statistically different ATP concentrations and qPCR counts of microbial abundance among sites evaluated at four spatial scales (1 m, 10 m, 100 m, and >1km), but apparent homogeneity of these sites at 'remote imaging' resolution (overall temperature, apparent moisture content, and regolith grain size). [1]

Field Sites: We performed the first characterization of soil communities in Maelifellssandur (63° 49.000' N, 19° 10.298' W). This recently deglaciated region north of Mýrdalsjökull is less well-studied and much less well-traveled than Fimmvörðuháls, decreasing the likelihood of anthropomorphic contamination. The region is covered at least to 15 cm depth in unconsolidated basaltic tephra that appears to be of nearly identical composition and grain sizes as the Fimmvörðuháls samples. It may be likely that ash from the Eyjafjallajökull eruption resurfaced both regions; however, this has yet to be confirmed.

Methods: Samples were collected from Maelifellssandur in 2015.[1] A triangular grid of sample locations spaced at 1 m, 10 m, 100 m and 1km intervals was established where the basaltic tephra is homogeneous based on visible color, morphology, and grain size. A triplicate sample set at 10 cm spacing was taken at each grid point. DNA was extracted from 0.25g of individual tephra sample using PowerSoil DNA Isolation Kit (MO BIO Laboratories Inc) according to manufacturer's instructions. High-throughput sequencing of PCR amplicons spanning the V3 and V4 hypervariable regions of the 16S rRNA geme tp assess the microbiome taxonomic composition. Amplicons were synthesized using 2x KAPA HiFi HotStart ReadyMix (Kapa Biosystems) with forward primer[2] (5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACA GCCTACGGGNGGCWGCAG-3') and reverse primer[2] (5' GTCTCGTGGGCTCGGAGATGTGTATAAGAGAC AGGACTACHVGGGTATCTAATCC-3'). Amplification was performed using denaturation at 95°C for 3 minutes, followed by 25 cycles of denaturation at 95°C for 30 seconds, primer annealing at 55°C for 30 seconds, primer extension at 72°C for 30 seconds, and a final extension at 72°C for 5 minutes. Amplicon products were verified using gel electrophoresis and purified using Diffinity RapidTip2 PCR purification tips (Diffinity Genomics, NY). Index PCR was performed to attach dual indices and Illumina sequencing adapters using the Nextera XT Index Kit (Illumnina). Thermal cycling condition for Index PCR was 95°C for 3 minutes, followed by 8 cycles of: 95°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds and final extension at 72°C for 5 minutes. Final products were verified using gel electrophoresis, purified using Diffinity RapidTip2 PCR purification tips and quantitated fluorometrically using the Qubit fluorometer (Life Technologies). Amplicons were pooled at equimolar concentrations and sequenced on an Illumina MiSeq using a 500 cycle kit. Sequencing data was analyzed using the QIIME pipeline[3].

Results: Based on high-throughput sequencing of 16S rRNA gene amplicons, *Proteobacteria* and *Actinobacteria* were the dominant microbial phyla representing over 50% of total sequences in all samples. However, a large number of other phyla (22) were also detected in this ecosystem. Although microbial richness did not vary significantly among samples (Chao1 index; $p>0.05$), the phylogenetic composition (weighted Unifrac metric) of the soil microbiome differed significantly among apparent homogenous site separated by >1 km ($p=0.028$), suggesting distinct microbial signatures despite apparent homogeneity. Future work will correlate microbial data with geochemical data to identify determinants of microbial community composition in this unique ecosystem, potentially helping guide future missions to detect analogous environments on Mars.

This work is part of Field Exploration and Life Detection Sampling for Planetary Analogue Research (FELDSPAR), on Facebook @FELDSPAResearch.

References: [1] Amador, E. S. *et al.* (2015) *Planet. Space Sci., 106* 1-10. Gentry, D. M. *et al.* (2017) *Astrobio.* in press. [2] Klindworth *et al.* (2013) *Nucleic Acids Res,* 41(1). [3] Caporaso *et al.* (2010) *Nature Methods*, 7(5) 335-336.