METHODS FOR EXTRACTING NUCLEIC ACIDS FROM MARS ANALOG REGOLITH. A. Mojarro[1], G. Ruvkun[2], M. T. Zuber[1], C. E. Carr[1,2,*], [1]Department of Earth, Atmospheric and Planetary Science, Massachusetts Institute of Technology, 77 Massachusetts Ave, Cambridge, MA, 02139, [2]Department of Molecular Biology, Massachusetts General Hospital, 185 Cambridge Street, Boston, MA, 02114, [*]E-mail: chrisc@mit.edu

Introduction: Biological informational polymers such as nucleic acids (e.g. deoxyribonucleic acid, or DNA), have the potential to provide unambigous evidence of life beyond Earth. Widespread synthesis of amino acids and nucleobases, the known building blocks of life, within the early planetary nebula [1-3] may have biased life on Earth towards utilizing nucleic acids. Life on Mars, if it ever developed, may have also adopted a similar medium and may have evolutionary ties to life on Earth due to the large amounts of meteoritic transfer that occured between Mars and the Earth during the late heavy bombardment period [4]. Our research group is developing a life-detection instrument that integrates automated nucleic acid extraction and nanopore sequencing, the Search for Extraterrestrial Genomes (SETG) instrument [5-9]. SETG will search for nucleic acids from extant or preserved life on Mars and genomic evidence of a Mars-Earth shared ancestry [4,10] aboard a future Mars rover or lander mission.

On Earth, metagenomic analysis of nucleic acids (DNA) extracted from environmental samples has been a powerful tool for identifying new and unique “unculturable” microorganisms [11]. However, complex matrices such as andisols (oxidized soils) have inhibitory side-effects when extracting nucleic acids due to competitive adsoption from silicates [12-14] and the production of destructive free radicals from Fe^{3+} and Fe^{2+} cycling in aqueous extractions [15]. These effects are especially problematic in low-biomass environments [16,17] and pose similar challenges for extracting nucleic acids from Martian soils [18]. Extensive literature has been published on how to mitigate soil-nucleic acid interactions and increase extraction yields [14,19]. Notable solutions, including grinding soils in liquid nitrogen [20], require involved steps and large amounts of reagents that would increase the complexity and footprint of an *in situ* nucleic acid extraction module on Mars.

In this study we utilize a miniature, two-step lysis and nucleic acid extraction PureLyse® module produced by Claremont Biosolutions, LLC (CBIO) to extract nucleic acids from Mars analog regolith. We report baseline DNA yields and outline the modifications required to attenuate soil interactions. Spores of *Bacillus subtilis* ATCC 6633 were processed in PureLyse® modules with and without JSC Mars-1A [21] and JSC 1A [22] Lunar regolith, well-characterized Mars aeolian and basalt simulants respectively.

Methods: Our PureLyse® nucleic acid extraction module is a CBIO OmniLyse® [23] mechanical cell disruption system coupled with binding and elution buffers that enables solid-phase nucleic acid extraction. Spores of *Bacillus subtilis* ATCC 6633 (Spore Suspension, SBS-08, NAMSA) were processed with and without (control) 50 mg of JSC regolith following the standard PureLyse® protocol and 2.1×10^8 spores or 902 ng of DNA. JSC regolith was sieved to <200 micron particles [24] and dry-heat sterilized for 48 hours at 130˚ C prior to any experiments [24]. Extracted DNA from two combined elutions (200 µl each) was quantified using a double-stranded, DNA-specific fluorometric assay (Qubit® dsDNA HS Assay Kit, Q32854, Invitrogen).

Spores of *B. subtilis* were then processed using a modified PureLyse® protocol with equal amounts of JSC regolith and spores. Samples of JSC Mars-1A regolith were degassed for 1 hour [25] and processed inside an anaerobic chamber (Atmosphere Bag, Z530212, Sigma) in the prescence of oxygen scavengers (AnaeroGen™ 2.5L, AN0025A, Thermo Scientific) and N_2 gas. An empiricaly determined amount of random hexamers (Random Primers, C1181, Promega), 150 ng, was added to samples of JSC 1A Lunar regolith and processed using the standard PureLyse® protocol. Elutions of JSC 1A were then cleaned using a genomic clean and concentrator (Genomic DNA Clean & Concentrator™-10, D4011, Zymo Research) to remove any annealed hexamers that may artificially increase DNA yields.

Results & Discussion: Baseline processing of *B. subtilis* spores with PureLyse® in water (control) produced a DNA yield of 15.6% (Figure 1). Previous trade studies of PureLyse® have demonstrated ~90% DNA yields from *Escherichia coli* [26]. We propose that resulting low yields from spores are the result of small acid-soluble proteins (SASPs) [27,28] that interfere with nucleic acid extraction. SASPs bind to DNA and furnish protection from heat, dessication, and UV radiation [29]. In short, SASPs are responsible for the resilience of *B. subtilis* spores. If life exists on Mars today, it is possible cells have adopted a similar mechanism for preservation, and consequently could make acquiring high yields of DNA problematic.

B. subtilis spores processed with JSC 1A lowered DNA yields to 12.9% and JSC Mars-1A lowered yields to 3.23% (Figure 1). Decreased yields with JSC 1A may be associated with DNA adsorption to silicates within the regolith [22], considering that silicates are often utilized in DNA purification assays. Reduced yields with JSC Mars-1A may be due to the production of hydroxyl free-radicals from iron cycling in our aqueous extractions [15]. Prior analysis of JSC Mars-1A regolith found a 3:1 ratio of Fe^{3+} to Fe^{2+} [15, 21].

In order to develop more effective methods of mitigating soil-nucleic acid interactions in the future, we have focused on resolving the dominant issues encountered for the regolith used in this study. Utilizing competitive binders, for example, ribonucleic acids (RNA), skim milk, and circular DNA, is a common practice for improving yields with andisols [14]. However, these additives are often inhibitory to downstream genomic analysis [19]. Instead we included random hexamer primers to JSC 1A, which increased DNA yields to 14.51% (Figure 1). These hexamers were removed from elutions with an extra cleaning step. In JSC Mars-1A regolith, presumed DNA destruction by hydroxyl free-radicals was migitated by removing dissolved oxygen from the PureLyse® reagents through degassing in an anaerobic chamber. We observed a yield increase from 3.23% to 6.79% in JSC Mars-1A using this methodology (Figure 1).

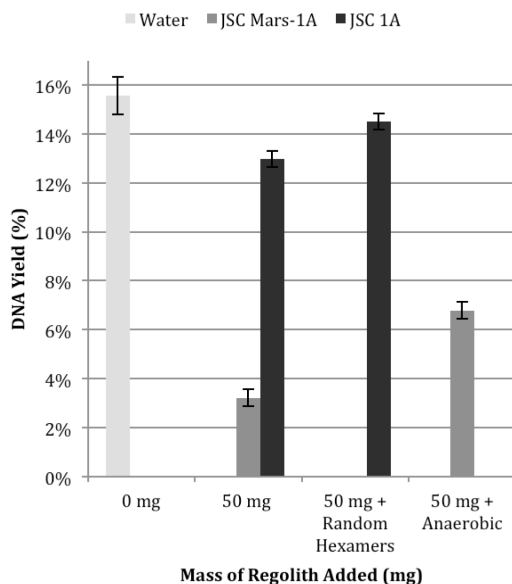

Figure 1: Nucleic acid extraction yields with a standard and modified PureLyse® protocol for *B. subtilis* ATCC 6633 from water, 50 mg JSC Mars-1A, and 50 mg JSC 1A (n=3) . DNA Yield (%) is defined as DNA Out / DNA In *100.

Conclusion: In this study we demonstrate the feasibility of extracting DNA from Mars analog regolith using a simple extraction module and provide an analysis of yield through different protocols. Future studies will focus on extracting DNA from various types of Mars analog regolith and testing an automated nucleic extraction system in the field. In addition, further research will investigate the role of SASPs in nucleic acid extractions and the purity of isolated DNA for downstream genomic analysis.

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