NANOPORE SEQUENCING FOR CHARACTERIZING THE MICROBIAL COMMUNITIES IN REGOLITH SIMULANT-BASED SOIL-LIKE-SUBSTRATES. L. E. Fackrell[1], A. C. Simpson[1], A. G. Palmer[2], N. K. Singh[1], and K. J. Venkateswaran[1], [1]Biotechnology and Planetary Protection Group, Jet Propulsion Lab, California Institute of Technology (laura.e.fackrell@jpl.nasa.gov); [2]Florida Institute of Technology, Department of Biomedical and Chemical Engineering and Science

Introduction: Regolith simulants are an essential tool commonly applied to astrobiology, exobiology, and space biology research. They provide an accessible substrate for multiple applications, from testing life detection technology [e.g., 1] to testing the potential for Earth microbial contamination of other planetary bodies from cleanroom or International Space Station (ISS) microbiomes [e.g., 2] to potential Biological In-Situ Resource Utilization (BISRU) Applications. The Oxford Nanopore Technologies (ONT) MinION™ also provides an accessible sequencing technology that is space-flight viable [2-3]. Herein we present preliminary results from a study intended to refine protocols for characterizing microbial communities in regolith-based substrates relevant to bioregenerative food systems (BFS) for future lunar and Martian habitats.

Experimental Approach: Plants grown in BFS in off-world communities may rely on a variety of material substrates that may incorporate regolith [4]. Plants may also produce a significant amount of waste from inedible plant material that needs to be recycled. One option is to use plant waste to make soil-like substrates (SLS) useful as growing mediums [5-7].

These systems will not be sterile environments, and it is essential to have the capacity to track the microbial communities that develop (intentionally or inevitably) so that appropriate protocols can be established for maintaining safe and reliable habitat conditions. Such materials also present a challenging medium for DNA extraction and sequencing technologies. This study provides preliminary results of nanopore sequencing of various SLS substrates (including regolith-based) and their components, the eventual goal is to develop reliable protocols and best practices appropriate for BFS-relevant substrates.

The Endogenous Community. Though it remains an open question whether the regolith on other worlds will contain endogenous organisms, the materials used to make regolith simulants certainly do. However, little work has been done to identify the endogenous microbial composition of different regolith simulants. Endogenous microbes may interfere with, outcompete, or enhance the action of intentionally-added microbes such as plant-beneficial bacteria and fungi, and if specific to a particular type of simulant could yield experimental results that would not be applicable to actual extraterrestrial regolith. Especially for studies where the large volumes of material needed make sterilization unrealistic, this is an essential baseline of information to have. Thus initial samples will refine protocols for soil DNA extraction, purification, library prep, and nanopore sequencing. It is expected that the regolith and plant materials may have lower biomass and/or other inhibitors that may require adaptation of the protocols as appropriate. Part of the purpose of these preliminary results is to develop appropriate protocols for DNA extraction and nanopore sequencing of these materials.

Changes to SLS Microbial Community. SLS are developed using a series of steps that alter inedible plant waste into a growing medium. Using inedible plant waste to make SLS is a viable means of producing plant growth mediums [5-7]. Still, it can have nonhomogeneous porosity and permeability issues, resulting in nonideal water uptake and distribution. Initial tests demonstrate that incorporating a mineral component (e.g., regolith) may improve these aspects [8]. Adding plant-growth-promoting microorganisms may also provide benefits such as increased nutrient availability or stress tolerance.

This study uses some of the preliminary test substrates (Figure 1) from that project as a starting point for refining protocols. The same protocols that will be adapted for identifying the endogenous community of regolith simulants will be applied to these test substrates and further adapted as needed. The results will provide appropriate protocols and initial insights into how the SLS microbial community changes at different stages of the SLS development process.

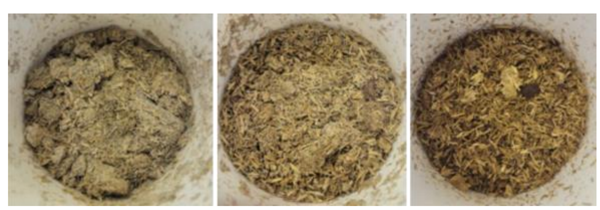

Figure 1: Partially matured SLS developed from plant material and lunar regolith simulant. These represent test samples used to help refine the protocols and provide initial insights.

Future Work and Potential Applications: The preliminary results of these studies provide appropriate protocols for future plant growth studies that will compare multiple characteristics of various regolith and non-regolith-based SLS, including changes to the microbial composition at different stages of SLS formation and subsequent use as a plant growth medium. It also provides protocols appropriate for similar materials that are relevant to space biology and astrobiology research in particular in helping to provide insights into endogenous communities of commonly applied regolith simulants.

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