MICROBIAL ECOLOGY OF THE JOHNSON SPACE CENTER METEORITE CURATION LAB AND ASSOCIATED INFRASTRUCTURE A. B. Regberg[1] A. S. Burton[1] C. L. Castro[2], S. E. Stahl[2] , S. L.Wallace[3], and F.M. McCubbin[1] [1]Astromaterials Research and Exploration Science Division, NASA Johnson Space Center, 2101 NASA Parkway, Houston TX 77058 , [2]JES Tech, 16870 Royal Crest, Houston, TX 77058, [3]Biomedical Research and Environmental Sciences Division, Johnson Space Center, 2101 NASA Parkway, Houston TX 77058, Email: aaron.b.regberg@nasa.gov

Introduction: The NASA Astromaterials Acquisition and Curation office maintains seven separate clean labs for storing extraterrestrial samples from the moon, meteorites, cosmic dust, asteroids, comets, solar wind particles and space exposed hardware. These clean labs are routinely monitored for elevated particle counts and trace metal contamination. In accordance with the advanced curation initiative[1], we initiated a microbiological monitoring program in order to understand what types of microbes are capable of surviving in these oligotrophic environments, and to determine whether or not they are capable of altering the composition of the samples stored therein.

We chose to start in the Meteorite Lab for the following reasons: 1) The cleanliness level of the lab would not be compromised by any of the sampling methods under evaluation. 2) The meteorite lab is an ISO class 6 clean room (many of the other labs are cleaner), so this study may provide us with a "worst case" for biological cleanliness in our labs. 3) Finally, the meteorite samples have been exposed to the terrestrial environment (Antarctic) for typically thousands of years before collection and hence the meteorites may host their own microbiological record. Consequently, we need to understand what is in the Meteorite Lab to establish a baseline for any future microbial studies of Antarctic meteorites.

Methods: Microbial samples were collected from three different surfaces in the Meteorite Lab. We collected samples from the laboratory floor, a table, and from inside a laminar flow bench used to process meteorites. Replicate, 300 cm^2 locations were sampled using dry foam swabs, dry polyester swabs, wetted polyester swabs, and wetted BisKit™ samplers. Wet polyester swabs were soaked with sterile water. The BisKit samplers were soaked with sterile PBS (phosphate buffered saline). After sampling, the swabs were processed in a sterile biosafety cabinet by adding 15 ml of PBS and vortexing at maximum power for 5-6 seconds to release the cells from the swab. Individual 0.2 ml. aliquots of PBS were plated onto TSA (Tryptic Soy Agar), BA (Blood Agar) and R2A (Reasoners 2 Agar) for bacterial growth, and PDA (Potato Dextrose Agar) and Saboraud Dextrose Agar for fungal growth. The plates were incubated for two to seven days before being inspected for bacterial and fungal growth, respectively. In order to compare our results to previously published studies all of the colony counts were normalized to a 25 cm^2 sampling area. The remaining PBS was retained for 16S rRNA gene sequencing. Isolated bacterial and fungal colonies were identified using the VITEK2[2] instrument or rRNA (16S gene for bacteria, 18S gene for fungi) Sanger sequencing.

Fungal colonies isolated from the laminar flow bench were selected for additional amino acid analysis. Samples from individual fungal colonies were transferred to glass ampoules with an organically clean Pasteur pipet (baked in air at 500 °C overnight). To each sample, 1 mL of ultrapure water (Millipore, 18.2 MΩ·cm, <3 ppb total organic carbon) was added. Ampoules were flame-sealed and incubated at 100 °C for 24 hours, after which they were dried under vacuum. Half of the sample was set aside to determine free amino acids content, whereas the other have was acid vapor hydrolyzed (6N HCl) for 3 hours at 150 °C to measure total amino acids (free and protein-bound). Samples were purified by cation exchange chromatography and analyzed by Ultra-Performance Liquid Chromatography with Fluorescence Detetction and Mass Spectrometry (UPLC-FD-MS)[3].

Additional microbial samples of opportunity were collected from the gaseous nitrogen filter (GN2) that had been used to filter N_2 for all seven curation labs. The filter was installed in 1979 and had not been opened prior to its decommissioning in 2017. These samples were collected using foam swabs and dry polyester swabs and were analyzed in the same manner as described above. One additional plate type was used on the filter samples. 1 ml of PBS was plated onto a TGA (Thyoglyocollate Agar) plate and incubated in an anaerobic chamber to encourage the growth of anaerobic bacteria and or fungi.

Results: Samples collected from the meteorite lab surfaces contained between 4 and 28 CFU (colony forming units) / 25 cm^2 (Fig. 1). Fungal colonies comprised 83 – 97% total CFU observed. The foam swab appeared to be the most universally efficient biomass collector. We cultivated 10.83 and 12.58 CFU / 25 cm^2 from the floor and table locations respectively. The BisKit worked well on the floor (13.83 CFU / 25 cm^2), but poorly on the table (0.42 CFU / 25 cm^2). The dry polyester swab was the only swab to successfully collect biomass from the flow bench (3.92 CFU / 25 cm^2).

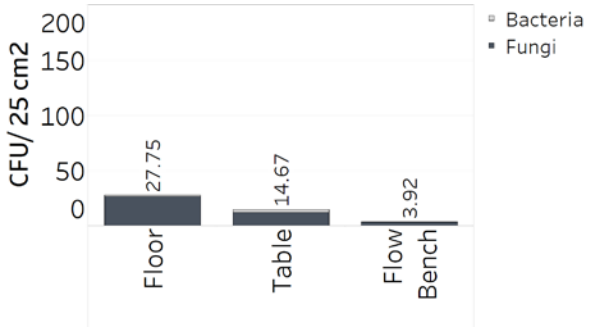

Figure 1: Fungal and Bacterial colony counts from three locations in the meteorite curation lab. Fungal counts (dark grey) are much higher than bacterial counts (light grey).

Surface area normalized samples collected from the N_2 filters contained between 0.2 and 184.1 CFU / 25 cm^2 (Fig. 2). Fungal colonies were less dominant but still prevalent in these samples and comprised between 8 and 100% of the cultured colonies. Anaerobic fungi and bacteria were also successfully cultivated from these samples.

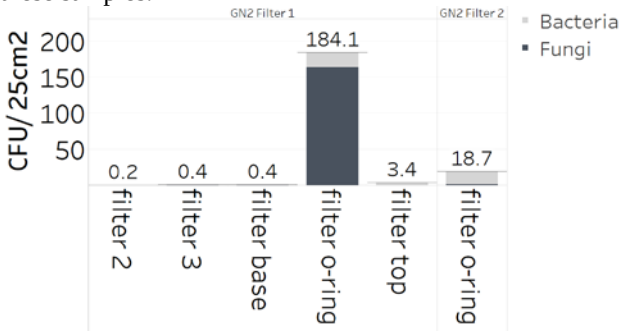

Figure 2: Fungal and Bacterial colony counts from locations in and on the N_2 filters used to purify nitrogen for curation use. Fungal counts (dark grey) are generally higher than bacterial counts (light grey).

Discussion: We cultivated viable fungal and bacterial colonies from oligotrophic environments that may have been isolated for as long as 20-30 years. The total number of CFU observed per unit surface area are consistent with culture based results from samples collected from JPL (Jet Propulsion Laboratory Space Craft Assembly Facility) and KSC (Kennedy Space Center Payload Hazardous Servicing Facility)[4]. However, the relative abundance of fungi contrasts with previous research which described nearly 100% bacterial CFU from aerospace clean rooms and associated infrastructure [4–6]. Other researchers have identified fungal DNA in clean room environments but have not reported culturing these organisms[7]. It is possible that the environmental conditions at the Johnson Space Center favor fungal growth more than those at the Jet Propulsion Laboratory or Kennedy Space Center. However, previous research did not focus on fungal cultivation in their choice of incubation media[4,6] so it is also possible that some fungal species were missed.

The presence of fungi in NASA curation facilities is particularly interesting since some fungal species are able to produce amino acids like Aib (α-aminoisobutryic acid) and Iva (Isovaline) that are often considered to be extra-terrestrial when identified in meteorites[8,9]. If these amino acids are identified in the environmental cultures from the meteorite lab it may be necessary to change and or improve our laboratory cleaning practices. Most of the identified fungal isolates belonged to the genus Penecillium. At least one member of this genus has been able to produce Aib in the lab[10]. The fungi cultivated from the laminar flow bench in this study are currently being analyzed for the presence and enantiomeric ratios of a broad suite of amino acids commonly found in meteorites, including Aib and Iva, to determine whether they could be possible sources of these compounds.

Conclusions and Future Work: The NASA meteorite curation lab as well as the filter used to purify N_2 are clean, but not sterile. In preparation for sample return missions with a prominent astrobiological component (e.g., OSIRIS-REx, CAESAR, Mars sample return), it will be very important to characterize and minimize the microbiology present in curation facilities.

We have begun routine microbial monitoring of the NASA curation clean labs in order to determine the amount and source(s) of microbial input into the labs. In addition to culture-based techniques, we will utilize next-generation DNA sequencing to build a more complete picture of the microbial ecology of these clean labs.

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