

FUNGAL EXPOSURE TO METEORITE THIN SECTIONS: DEVELOPING AN EXPERIMENTAL TO OBSERVE BIOGEOCHEMICAL CHANGES

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Introduction: The Astromaterials Acquisition & Curation Office maintains collections of meteorite samples collected as part of the Antarctic Search for Meteorites (ANSMET) program. The chief goal of the curation department is to maintain these meteorites in pristine condition. The Astromaterials Research and Exploration Science (ARES) Directorate has implemented a microbial monitoring program for the meteorite collections that has resulted in the isolation of >100 fungal isolates [1], however it is currently unknown if these isolates could present danger to the collections through bioweathering or secretion of organic compounds. We grew a strain of *Fusarium oxysporum* isolated from nitrogen gas filters feeding the Meteorite Lab nitrogen gas in the presence of a H5 meteorite thin section to determine if this fungus has the capability of altering the mineral structure of this common meteorite.

This first trial was to determine and understand the effects of *Fusarium oxysporum* growth on the iron content within H5 chondrites and evaluate what additional components are needed in the development of future trial runs. This experiment determined new considerations for handling samples, the nature of microscopic scans before and after the incubation period, and the quality of the samples utilized for the experiment. The results of this experiment were promising and warrant further investigation with a more refined process and timeline.

Materials and Methods: Thin Sections of H5 Chondrites ALHA-78085 and RKPA-79004 were mounted with polymer to circular glass slides prior to this project. Both samples were placed in a WITec alpha300 R imaging Raman microscope and mapped under reflected and 488nm blue light for ALHA-78085 and 633 nm red light for RKPA-79004 to determine their petrology. These scans were conducted prior to incubation with *F. oxysporum*. Both thin sections were also examined through JEOL 8530F Electron Probe Microanalysis (EPMA) using energy dispersive spectroscopy (EDS) for elemental mapping.

Experimental Design and Process. For this study, we prepared Sabouraud (SAB) media, a substance with a slightly acidic pH (~5.6) and high sugar content (40g/L dextrose). The decision to use Sabouraud media for this experiment was predicated on the knowledge that *F. oxysporum* was able to grow in SAB media in anoxic conditions [3].

Following the preliminary data collection, ALHA-78085 was placed in a sterile sample container in SAB media and inoculated with *F. oxysporum*. RKPA-79004 served as the negative control sample with no inoculation. We placed the samples within a 90% N₂-5% H₂ glove box for a period of 13 days at 30°C with rotational mixing to encourage strong fungal growth. After the incubation period, the thin sections were removed from the SAB media, scanned with the reflected light and Raman microscope, and then cleaned with ethanol before a final EPMA scan.

Results: Adhesive on Thin Sections. ALHA-78085 and RKPA-79004 had to be sterilized with 70% ethanol to remove any excess bacteria on the thin section surface. The ethanol cleaning of RKPA-79004 loosened the thin section and it detached from the glass slide in the sterile container during the incubation period.

Fluorescence in Raman Scan. We used Raman microscopy to accurately map the mineralogy of ALHA-78085 prior to the incubation experiment. The meteorite contained olivine, pyroxene, magnetite, Fe-metals, and multi-molecular carbons. -79004 fluoresced when exposed to the 633 nm laser and thus

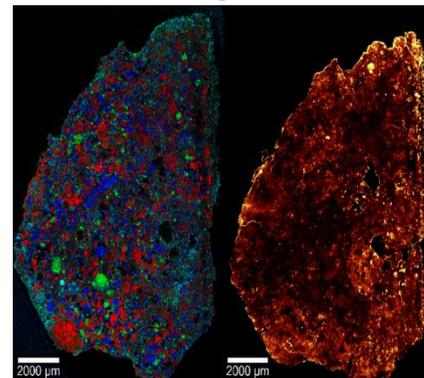


Figure 1: Image on the left shows the Raman mineral maps of pyroxene, olivine, and multi-molecular carbon (MMC) within ALHA-78085 prior to incubation with *F. oxysporum*. Image on the right depicts an attempt at determining the fungal coverage after incubation; however, the wavelength used to locate fungal coverage was extremely similar to one type of background fluorescence.

we could not obtain Raman spectra. It is unclear if this was due to the type of adhesive on the thin section or some other unknown factor. After the incubation period, ALHA-78085 also fluoresced when exposed to the laser which prevented us from gathering any Raman data (see Fig. 1). We suspect that the electron beam from EPMA scan before the incubation period damaged the mineral surface causing it to fluoresce under 488 nm light.

Elemental analyses of the thin section showed fungal hyphae attached to the thin section and possibly embedding into the section itself. Iron mapping showed significant differences after fungal growth with several chondrites severely altered or missing. The matrix was also altered with several elements (P, S, Ni) showing significant changes or reduction in the meteorite. Also observed were several new fractures in the thin section that may have been caused by hyphal growth and dissolution of minerals

Four days after the start of the incubation period, the media in the container with RKPA-79004 became clouding indicating contamination from an unknown microorganism. We observed filamentous bacterial chains that only contaminated RKPA-79004 and not ALHA-78085. This contamination is likely the result of a form of bacillus left of the thin section's surface even after being cleaned with ethanol, rather than the result of the SAB media which was autoclaved prior to incubation.

Future Work: Future experiments should consider using freshly prepared thin sections in order to account for the quality of samples and adhesives used to secure them to the glass surface. The damage to ALHA-78085 from the electron beam during the first EPMA scan was due to the unusually high energy setting for the beam. Ordering the production of fresh samples, possibly at 100µm or thicker to minimize E-beam damage, with epoxy or another non-reactive adhesive to ensure proper quality control prior to data collection.

We hope to determine the molecular mechanisms for these observations as we learn more about the biochemistry of the *Fusarium oxysporium* isolate. We have sequenced the genome of the fungus and hope to learn how Ph and secreted natural products such as siderophores contribute to the weathering of minerals

in H5 meteorites. We also hope to determine the mineralogical changes this fungus can produce in whole, uncut meteorites to help us understand how biological contamination in the cleanroom environment can alter astromaterial minerals after collection.

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References: [1] Davis, R. et al (2018) *Isolation and Monitoring of Cleanroom-Associated Microbial Contaminates From Geological Collections*. [2] Tsuyoshi A. et al. (2007) *Bioscience, Biotechnology, Biochemistry*, 71. 2402-2407.