MICROBIAL DIVERSITY ANALYSES OF TERRESTRIAL SHOCKED BASALT AND SHOCKED BASALTIC SOIL: IMPLICATIONS FOR PANSPERMIA AND FUTURE EXOBIOLOGY MEASUREMENTS
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Summary: Microbes were found in three samples from Lonar Crater, India: an unshocked paleosol, a shocked soil, and a shocked basaltic rock. Characteristics of the microbes are described here (on 3 tables) and implications for panspermia and biological measurements by rovers are discussed.

Introduction: Astrobiology is a primary goal of the Mars Exploration Program. The payload for every rover includes some instrumentation whose purpose is to measure the water or organic content of Martian soils and rocks, with the instrumentation getting more and more sophisticated in time. The next step will likely be detailed measurements of biological activity, but decisions on what will be measured and what the instrument results mean will have to be considered carefully. This work represents a preliminary microbial diversity analysis for constraints on what properties of organics must be measured to avoid ambiguous results.

In addition to on-surface measurements by rovers, this preliminary work has implications for panspermia in that shergottites (Class 2 shocked basalts from Mars) and recent sedimentary breccias from Mars [1] are basalts or breccias delivered from Mars. These may have clues to Martian exobiology without the need for Mars missions. All of the shergottites are shocked 25-45 GPa just as the shocked soil sample from Lonar used in this study [2]. Rather than first describing the samples, and then the microbial results, the three samples are described preceding the table showing the microbial results.

Methodology: Analyses of the three samples included polymerase chain reaction (PCR) amplification and RNA sequencing of the 16S ribosomal gene. These analyses return microbial identification and diversity. The 16S or 18S functional gene is generally used for metagenome analyses. The samples were amplified using fusion primers and then sequenced. The data generated is then denoised and compared to several databases of curated sequences of 6 broad assays: bacteria, fungi, archaea, mycobacterium, algae, and cyanobacteria. The goals of the analyses are to see what microbes are in the three samples, relate their microbial characteristics to the geologic history or details of their “host” sample, and make comparisons between the two shocked and one unshocked sample. This is relevant given that the shocked soil is roughly the shocked equivalent of the unshocked soil.

Results: 202 microbes were found in the shocked soil, with 211 microbes found in the shocked basalt sample. The unshocked paleosol had just 12 microbes. Data comparing which microbes the three samples have in common is too large to list here; here, we list the microbes found with at least 5% abundance in Tables 1 to 3 below. Future work will address the least abundant microbes in the samples. Each table comes after a brief description and petrography of the samples.

Table 1: Microbes with at least 5% abundance in shocked basalt sample

<table>
<thead>
<tr>
<th>Microbe</th>
<th>Abundance</th>
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<tbody>
<tr>
<td>solirubrobacter</td>
<td>8.8%</td>
</tr>
<tr>
<td>rubrobacter</td>
<td>7.2%</td>
</tr>
<tr>
<td>ustilago maydis</td>
<td>5.6%</td>
</tr>
</tbody>
</table>

Shocked basalt: LC09-275 was chosen for analyses due to its shock level of Class 2 [3,4], matching the shergottites [2], but also due to obvious hematite and decompression cracks (seen here [5]) that we suggest may spur microbial growth and access to nutrients. Whether or not the microbes survived the shock ~670 ka [6] cannot be answered by this work. We are currently looking into a method to place age constraints on the microbes.

Figure 1. Petrography of a hematite-rich Class 2 shocked basalt in PPL and CPL. Note that needles of labradorite are isotropic in CPL, indicating maskelynite.

hematitic Class 2 shocked basalt LC09-275

<table>
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<td>solirubrobacter</td>
<td>8.8% of microbial activity</td>
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<td>rubrobacter</td>
<td>7.2%</td>
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<tr>
<td>ustilago maydis</td>
<td>5.6%</td>
</tr>
</tbody>
</table>

- found in soil
- aerobic
- radiotolerant
- found in hot springs, thermal environments, and desert soils
- fungi or yeast that inhabits dead soil or plant environments
- dimorphic
- pathogenic to plants, especially corn
- teleiospores allow the fungus to survive winter and drought; prefers dry conditions 78°-93° F
- occurs in soils with high nitrogen contents such as manure
Paleosol: LC09-PS-261 was collected under the lithic breccia layer in the Lonar ejecta. Paleosol represents the uppermost soil in the Deccan region before the Lonar impact event deposited ejecta on top of it. The soil texture of the paleosol is shown below.

**Figure 2.** Petrography of paleosol LC09-PS-261 in plane polarized (PPL) and cross polarized light (CPL).

<table>
<thead>
<tr>
<th>paleosol LC09-PS-261</th>
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<td>total microbes: 12 ranging from 3.8% to 11.2%</td>
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</tbody>
</table>

- tepidimonas spp. [11.2%]
  - gram-negative
  - strictly aerobic
  - oxidase and catalase-positive
  - rod-shaped
  - slightly thermophilic bacteria

- sphingobium yanoikuyae [8.9%]
  - found in aquatic environments
  - depletes various bisphenols in aquatic env.

- acinetobacter genoms. 3 [8.8%]
  - common in soils and water
  - pleomorphic oxidase-negative
  - non-motile bacteria

- chlorochromatium / chlorobium [8.5%]
  - genus of green sulfur bacteria
  - photolithotrophic
  - lives in strictly anaerobic conditions below water surface

- burkholderia ubonensis [7.6%]
  - bacteria found in soil, groundwater worldwide
  - all temperatures, including 7°F in Arctic
  - human and plant pathogen

- ralstonia picketti [6.2%]
  - gram-negative, rod-shaped bacteria
  - found in moist environments
  - oligotrophic; capable of surviving with very low concentrations of nutrients
  - some strains survive in environments with high metals such as Cu, Ni, Fe, and Zn

- arthrobacter spp. [5.8%]
  - soil bacteria
  - several species can degrade pesticides
  - gram-positive obligate aerobes

Shocked soil: A gray, frothy sample was found in the suevite / impact melt-bearing breccia unit. The density is unlike basalt and resembles that of popcorn. Upon petrographic examination (Figure 3), this sample LC09-316 was found to be a shocked soil due to its similarity to the petrography of paleosol (Figure 2) in that tiny, comminuted fragments of augite and labradorite are found in a soil-like matrix, but with flowing carbonate and glass.

**Figure 3.** Petrography with 5X magnification of shocked soil LC09-316 in PPL (left) and CPL (right).

<table>
<thead>
<tr>
<th>shocked soil LC09-316</th>
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<tr>
<td>total microbes: 202 ranging from &lt;.002% to 14.9%</td>
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</table>

- rubrobacter spp. [14.9% of microbes detected]
  - radiotolerant
  - found in hot springs, thermal environments, and desert soils

- acidimicrobiales spp. [10.6%]
  - ferrous iron-oxidizing bacteria
  - thermophilic, acidophilic

- Solirubacter spp. [7.5%]
  - aerobic; found in soils

- bosea spp. [6.3%]
  - amoeba-resisting bacteria

- leptospirillium spp. [5.5%]
  - iron-oxidizing bacteria
  - important role in bioleaching, biooxidation
  - obligate aerobes

**Discussion:** Several findings suggest that more samples should be analyzed for comparison. Only 12 microbes in the (unshocked) paleosol suggest that its location under ~5 m of lithic breccia, only recently made available for the field geologist to sample at a quarry [7,8], might not be conducive to microbes. Further, the shocked basalt sample used here was a float/talus rock [5] that may have had some soil contamination due the characteristics of the microbes present. The precious, delicate shocked soil from the suevite layer requires more study to determine if the microbes survived shock.


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