

Terrestrial microbes in martian and chondritic meteorites

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Introduction: The best extraterrestrial analogs for microbiology are meteorites. The chemistry and mineralogy of Asteroid Belt and martian (SNC) meteorites are used as tracers of processes that took place in the early solar system. Meteoritic falls, in particular those of carbonaceous chondrites, are regarded as pristine samples of planetesimal evolution as these rocks are primitive and mostly unprocessed since the formation of the solar system 4.56 billion years ago. Yet, questions about terrestrial contamination and its effects on the meteoritic isotopic, chemical and mineral characteristics often arise. Meteorites are hosts to biological activity as soon as they are in contact with the terrestrial biosphere, like all rocks. A wide biodiversity was found in 21 chondrites and 8 martian stones, and was investigated with cell culture, microscopy techniques, PCR, and LAL photoluminetry. Some preliminary results are presented here. The sample suite included carbonaceous chondrites of types CR, CV, CK, CO, CI, and CM, from ANSMET and Falls.

Past studies documented the alteration of meteorites by weathering and biological activity [1]-[4]. Unpublished observations during aqueous extraction for oxygen isotopic analysis [5], noted the formation of biofilms in water in a matter of days. In order to address the potential modification of meteoritic isotopic and chemical signatures, the culture of microbial contaminating species was initiated in 2005, and after a prolonged incubation, some of the species obtained from cell culture were analyzed in 2006. The results are preliminary, and a systematic catalog of microbial contaminants is developing very slowly due to lack of funding.

Methods: The primary method was cell culture and PCR.

Chondrites. Chondritic meteorite fragments were obtained by breaking stones of approximately one gram in sterile mortars. The core of the rocks, presumably less contaminated than the surface, was used for the present microbial study, and the remaining fragments of the samples were used for amino acid and isotopic analyses [6]. Some samples were fragments of dried and wet meteorites isolated in centrifuge tubes after a 10-day water extraction. Sabouraud Dextrose (dilutions 1:10 and 1:1000), Bacto Agar, LB Broth Miller (dilutions 1:10 and 1:1000), and R2A agar (1:1 and 1:1000), were autoclaved and cooled in culture plates inside a clean hood for cell culture. Some controls retained sterile moist agar still adhering to the perimeter of the plates for up to 18 months, and validated the sterile technique. Cell culture, PCR and microscopy documented a diversity of archaea, prokaryotes and eukaryotes in these samples [7].

The plates displaying microbial growth at room temperature after 6 weeks or less were used to produce streak plates and isolate colonies of individual species for long term freezing in Eppendorf tubes. Any plate with biological growth along the perimeter of the plate was discarded. The plates without microbial activity after 6 weeks were stored in a fridge for 18 months.

Control plates, exposed to the clean hood, laboratory room, used gloves, and weighing paper used in the analyses, sustained the prolonged storage with no sign of microbial activity that could be related to the analysis method. Dust grains and water extracts from the meteorites were spread on agar surfaces in cell culture Petri dishes in a clean hood.

SNC samples. In early 2005, the surface of SNC stones in the USNM curation facility were brushed with sterile swabs. Fallen dust grains were collected on weighing paper and isolated in sterile tubes. The sample suite included Zagami USNM 6545, Lafayette USNM 1505, Los Angeles USNM 7052, Shergotty USNM 321, Nakhla USNM 5892, Nakhla USNM 426 (117.4 g) and Nakhla USNM 426 (18.2 g), and Chassigny USNM-MNHN 2524. The controls, worker's gloves, blank swabs, and weighing paper exhibited no microbial activity in subsequent months. The cell culture was conducted with Sabouraud Dextrose and R2A only, by deposition of dry grains onto the surface of agar in culture plates that were incubated at 20 Celsius 2 weeks, then at 35 Celsius 3 days, and finally at 4 Celsius for 18 months. Limulus Amoebocyte Lysate (LAL Assay) measurements of the swabs [7] revealed low biological activity in all SNCs, except in a small piece of Nakhla USNM 426 (18.2 g) (activity below detection limit) and Chassigny (data unavailable). The LAL technique assesses gram negative bacterial equivalents.

Culture sample material was recovered from the agar plates using sterile pipette tips.

The material was added to 100 uL sterile water. This material was boiled for 15 min. in a water bath to lyse the cells and inactivate enzymes. 10 uL of the boiled lysate was used as template in PCR. Universal bacterial primers (27F and 1492R) were used to amplify a major portion of the 16S rRNA gene. PCR product was purified using a Qiagen MinElute PCR Purification kit and then sent for sequencing.

Results and Comments:

GRA 95229. Biological growth out of dust grains deposited on the agar surface was visible with the naked eye, and occurred after incubation in a fridge for 10 months.

Leoville. Biological growth occurred at the contact of a dust grain and the agar surface after 1 month. It continued to expand outside a dust grain deposited on Sabouraud Dextrose 1:1000, while stored at 20 Celsius for 12 months. The fan shaped outgrowth reached about 1 cm on either side of the meteorite grain and dried. DNA was recovered, and PCR products yielded sequences of a *Bacillus* spp. *Bacillus* is a common soil bacterial genus, and the close sequence relatives of this isolate were no exception. They were rock or soil *Bacillus*, and have been found in Allende [4].

EET 87770. Rock fragments were wet for 8 months (Millipore water) in a sterile centrifuge tube, and were used to make a spread plate that dried over a period of 10 months. The yellow dried colonies yielded good PCR product and the sequences were compared to other GenBank sequences using the BLAST program. The closest matches were in the genus *Microbacterium*. Soil and plant isolates were close relatives by sequence comparison.

Los Angeles. After 11 months of incubation in a fridge, a yellow colony grew at the center of a culture plate of Los Angeles dust grains (1:1000 R2A). There was no cell activity in the other agars. A DNA extraction yielded no usable results [7]. Sequencing was not performed because the culture plate became contaminated with outside organisms that overtook the colony of interest.

Conclusions: The sequences for EET 87770 and Leoville were of a good quality and the sequence reads were long, so the data are clear that these are typical soil and/or plant-related bacteria commonly found in Earth habitats. Microbial species present in a dozen chondritic samples from isolates are not yet identified, and the contaminant in Los Angeles needs to be recovered. In addition, isotopic analyses of samples with various amounts of microbial contamination could help quantified isotopic impact of microbes on protoplanetary chemistry in these rocks.

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[4] Whitby, C. et al. (2000)) *LPS XXXI*, Abstract #1732. [5] Airieau, S. A. et al (2005) *Geochim. Cosmochim. Acta*, 69, 4166-4171. [6] Unpublished data, with H. J. Cleaves, A. Aubrey, J. Bada (Scripps Institution of Oceanography), M. Thiemens (UC San Diego) and M. Fogel (Carnegie Institution of Washington). [7] Unpublished data, with A. Steele (CIW), and N. Wainwright (Marine Biological Laboratory).

Acknowledgements: Lisa Welleberger for access to SNC samples at USNM; Ralph Harvey for organizing ANSMET; Denise C. Thiry and Andrew Steele for long term storage of samples, Norm Wainwright for LAL measurements. A small portion of this work was funded with a NASA Cosmochemistry grant, (P. I. Thiemens).