

OPTICAL RECOGNITION OF 830 MILLION YEAR OLD MICROORGANISMS TRAPPED IN BEDDED HALITE: IMPLICATIONS FOR FUTURE RETURN SAMPLES FROM MARS. S. I. Schreder-Gomes and K. C. Benison, West Virginia University, Department of Geology and Geography, Morgantown, WV 26506.

Introduction: Recent studies have shown that microorganisms can survive and thrive in conditions well outside of what is largely considered habitable, including extremely acid-saline environments (e.g. [1]). Microorganisms living in these extreme environments can be trapped in rapidly growing saline minerals as they precipitate from brines [2, 3]. In this manner, microorganisms can be preserved within fluid inclusions and remain unaltered for millions of years.

Recent studies have made petrographic observations of microorganisms and organic compounds in fluid inclusions in modern, ~30 Ka and ~270 Ma halite, as well as in modern gypsum [2, 3, 4, 5, 6, 7, 8]. Non-destructive studies, using petrography and laser Raman spectroscopy to document prokaryotes, eukaryotes such as *Dunaliella* algae, and identify organic compounds such as beta carotene and long-chain waxes [4, 5, 6, 9].

This ongoing study surveys Neoproterozoic (~830 myo) halite for evidence of microorganisms trapped in primary fluid inclusions in halite. The Empress 1A core from the Browne Formation of Western Australia contains bedded halite, bedded gypsum/anhydrite, and hematite-rich mudstones and sandstones. Bedded halite was deposited in shallow saline brines [10]. These rocks offer a rare chance to observe and characterize preservation of microorganisms and organic compounds.

This research is relevant to the astrobiology community because it may serve as an analog for martian chemical sediments. The methods used here may prove useful for examination of samples returned to Earth from Mars in the future.

Methods: Here, we highlight observations from five halite chips from 1520 m depth in the Empress 1A core. A razor blade was used to break halite chevron crystals into chips <1 cm in length and width. Halite chips were polished with ultra-fine grit sandpaper to ~1 mm, a thickness that is optimal for petrographic study. We examined halite chips with Olympus microscopes at 6.3 – 2000 x magnification range, using plane transmitted, polarized, reflective, and UV-vis (330 nm and 385 nm) light. With low magnification and plane transmitted light, growth bands of primary fluid inclusions were identified. In contrast, use of high-magnification, long-working distance objectives allowed for micron-scale resolution of liquid, gas, and solid phases within individual inclusions. Because the microscopes focus into crystal depths, this method is non-destructive and inclusions are protected from any contamination.

Results and Interpretation: Petrographic observations made from core samples from one interval of bedded halite at 1520 m depth displayed exceptional abundance and preservation of microorganisms in primary fluid inclusions. These primary fluid inclusions are cubic to subcubic, are oriented parallel to one another along growth bands, and commonly range in size from 5 μm – 30 μm . Secondary inclusions are irregularly shaped, generally larger, may cut across bands of primary fluid inclusions and/or crystal boundaries, and form from the alteration of halite. Secondary inclusions were noted but not examined for suspect microorganisms. All suspect microorganisms in this study are from primary fluid inclusions.

Criteria for distinguishing suspect microorganisms from inorganic solids include size, shape, and color. Suspect prokaryotes are the most common microorganisms observed in the Browne Formation halite. They are generally ~0.5 μm to ~1 μm cocci, are bright with high relief, and can appear white or pale orange to pale blue when viewed under plane transmitted light. In contrast, suspect algae are 3 - 5 μm spheres that are pale orange, pale yellow, or clear (Fig. 1A). Some appear to have a dimpled surface texture, while others have a 'halo' of a viscous liquid, such as glycerin or beta-carotene. Organic compounds, besides the viscous liquid halos, appear as irregular, blobby shapes that are colorless to pale yellow. They are often observed in the same fluid inclusions as suspect microorganisms.

Using other light sources, such as UV-vis and polarized light, also aid in recognizing suspect microorganisms. In modern microorganisms, the color of UV fluorescence can be used as a criterion to distinguish types of microorganisms and organic compounds. For example, prokaryotes commonly fluoresce green-blue and eukaryotes commonly fluoresce blue in response to UV-vis light [11]. However, fluorescent response may be of different color, fainter or lacking for older microorganisms [4].

In this study of 830 Ma halite, bright white, pale yellow-gold, and pale blue fluorescence was associated with algal and prokaryote suspects (ex: Fig. 1B). We noted that focus of UV-vis light at high magnifications seems to vary by specific microscope objectives. Optimal fluorescent response was detected at 960 x magnification. However, the ~0.5 μm to ~1 μm prokaryotes are best seen at 1250 – 2000 x magnification. We have observed that prokaryotes exhibit fainter fluorescent response than do larger eukaryotes; this is presumed due to their small size.

Therefore, assigning specific fluorescent response to individual microorganisms, specifically prokaryotes, can be challenging.

Polarized light can be helpful in distinguishing minerals from microorganisms and organic compounds. Some tiny crystals are entrapped as solid inclusions along growth bands, and as “accidental” daughter crystals within primary fluid inclusions in Browne Formation halite. These minerals, if they are not halite or another isotropic mineral, will usually exhibit birefringence, eliminating them as possible microbial suspects.

In the halite chips studied from the 1520 m interval, there were microorganisms present in <40% of fluid inclusions. If present, it was most common to see one or two suspect microorganisms in an individual fluid inclusion (Fig. 1C). Rarely, there were occurrences of as many as ten microorganisms in the same fluid inclusion.

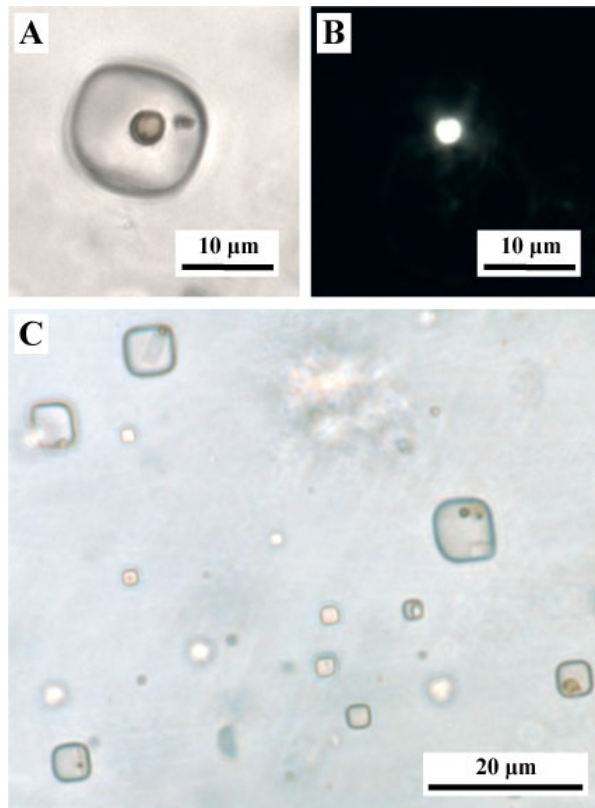


Figure 1. Suspect microorganisms in primary fluid inclusions in halite. A) Suspect algae in plane transmitted light. B) Same view as A, but in UV-vis light. C) Plane light view of multiple fluid inclusions containing suspect microorganisms and daughter crystals.

Implications: These results show that microorganisms and organic compounds can be preserved in fluid inclusions for hundreds of millions of years. This suggests that there is potential that any microorganisms that may have existed on Mars in the ancient past may have been trapped as microfossils in saline minerals. This may be the best known style of preservation of ancient extremophiles.

These results prove the method is a viable option for non-destructive investigation of some future returned martian samples. Optical microscopy of martian chemical sediments and other aqueous-precipitated minerals is a necessary method that cannot be done by rover and requires sample return. Fluid inclusion petrography allows for distinction between primary and secondary fluid inclusions. Primary fluid inclusions are important because they are original surface water remnants, and as such, are microhabitats. In contrast, secondary fluid inclusions record diagenetic fluid history.

Optical petrography should be considered a first analytical step for martian returned samples. The optical recognition of microorganisms and organic compounds in samples from Mars would be an important non-destructive preliminary investigation prior to further analyses, such as laser Raman spectroscopy, culturing, and metagenomic studies.

Acknowledgments: Funding provided by NASA Exobiology research grant 80NSSC18K1286 and NASA Mars 2020 research grant 80NSSC20K0235 to KCB, and NSF/GSA Graduate Student Geoscience Grant # 12887-20, which is funded by NSF Award # 1949901 and West Virginia University Heald Fund Research Grant to SSG.

References: [1] Zaikova E. et al. (2018) *Extremophiles*, 22, 367–379. [2] Lowenstein T. K. et al. (2011) *GSA Today*, 21, 4–9. [3] Schubert B. A. et al. (2009) *Astrobiology*, 9, 467–482. [4] Benison K. C. (2019) *Front. Environ. Sci.*, 7, art. 108. [5] Benison K. C. and Karmanocky F. J. (2014) *Geology*, 42, 615–618. [6] Conner A. J. and Benison K. C. (2013) *Astrobiology*, 13, 850–860. [7] Schubert B. A. et al. (2010a) *Environ. Microbiol.*, 2, 440–454. [8] Schubert B. A. et al. (2010b) *Geomicrobiol. J.*, 1, 61–75. [9] Winters Y. D. et al. (2013) *Astrobiology*, 11, 1065–1080. [10] Bernau J. A. (2017), *M.S. Thesis, West Virginia U.* [11] Mormile M. R. and Storrie-Lombardi M. (2005) *Astrobiol. & Planetary Missions, Proc. of SPIE*, Vol. 5906