CRYOGENIC SILICIFICATION OF MICROORGANISMS IN HYDROTHERMAL FLUIDS. M. G. Fox-Powell[1], A. Channing[2], D. Applin[3], P. Mann[3], E. Cloutis[3], L. J. Preston[4] and C. R. Cousins[1]; [1]School of Earth and Environmental Sciences, University of St Andrews, Irvine Building, North Street, St Andrews, UK (mgfp@st-andrews.ac.uk), [2]School of Earth and Ocean Sciences, Cardiff University, Cardiff, Wales, UK, [3]Department of Geography, University of Winnipeg, Winnipeg, Manitoba, Canada, [4]Dept. Earth and Planetary Science, Birkbeck, University of London, Malet St., Bloomsbury, London, UK.

Introduction: The delivery of hydrothermal fluids into low-temperature planetary surface environments is a common process in the solar system. Opaline silica has been observed on Mars [1], and shortlisted landing sites for the NASA Mars 2020 rover include relic hydrothermal systems bearing opal-A deposits. Recent detections of colloidal silica within the cryovolcanic plumes on Enceladus offer the strongest evidence to date for ongoing hydrothermal activity on another celestial body [2], and further data suggests that the liquid water sourcing these plumes has conditions suitable for biological methanogenesis [3].

The well-documented silicification of microbial biosignatures on Earth makes opaline silica an attractive target material in the search for extraterrestrial life. However, the sub-zero surface temperatures which characterize other planetary bodies mean that silicification mechanisms will differ to those commonly studied on Earth. This has direct implications for the preservation of any resident microorganisms.

When frozen, silica-rich fluids precipitate cryogenic opal-A (COA) within ice-bound brine channels [4]. The result is the formation of particles with distinctive morphologies defined by the physical dimensions of the brine veins (Fig. 1A). The fate of microorganisms during this process is presently unknown. Previous work has identified silicified microorganisms in association with natural COA particles from Iceland [5], however without a demonstration of silicification under entirely cryogenic conditions, it is presently impossible to discriminate biomorphic structures that formed during freezing from those that were formed subsequently under different (e.g., non-freezing) conditions. Here we combine an examination of natural samples from Yellowstone National Park and Iceland with experimental cryosilicification of microorganisms to investigate the nature of preservation (or lack thereof) for microorganisms and associated biosignatures during freezing of silica-rich hydrothermal fluids.

Experiment: Four strains of microorganism were selected to capture morphological, phylogenetic and metabolic diversity, and grown to stationary phase in pure culture. Strains included (i) the thermophilic sulfate-reducing bacterium *Thermodesulfovibrio islandicus* DSMZ-12570, (ii) the thermophilic methanogenic archaeon *Methanoculleus thermophilus* DSMZ-2373,

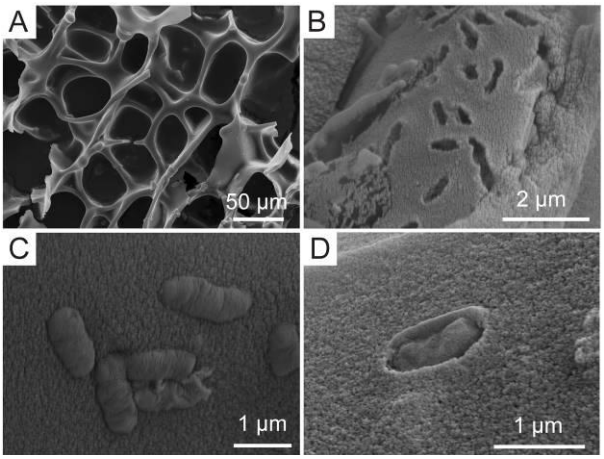

Fig. 1: *SEM images of experimental and natural COA particles. (A) Intact experimental COA lattice. (B) Experimental COA branch containing cell casts of* R. palustris. *(C) Experimental COA sheet exhibiting fully encased cells of* R. palustris. *(D) Cell cast of* M. thermophilus *on an experimental COA branch, containing deflated cellular remnants.*

(iii) the filamentous anoxygenic phototroph *Chloroflexus aurantiacus* DSMZ-635, and (iv) the photoferrotrophic bacterium *Rhodopseudomonas palustris* TIE-13. Cells were harvested by centrifugation, washed and added to a synthetic hydrothermal fluid (500 ppm Si, pH 7.7), maintained at 55° C. All experiments were then immediately placed into a -20° C freezer to initiate COA precipitation. After 24 hrs, frozen samples were defrosted, air-dried, rinsed gently with ultrapure water and prepared for analysis.

Microscopic analyses. COA were observed under optical microscopy and subsequently gold-coated for SEM analysis at the University of Edinburgh. SYBR Gold (Invitrogen) was used to stain COA suspensions prior to fluorescence microscopy.

Spectroscopic analyses. Visible-Short-wave infrared reflectance (vis-SWIR) and Raman spectroscopy were carried out at the University of Winnipeg. Fourier-transform infrared (FTIR) spectroscopy was carried out at Birkbeck, University of London.

Results: Our microscopic observations reveal that microbial cells are incorporated into COA particles, becoming completely or partially encased within the

colloidal silica matrix (Fig. 1B-D). The mode of preservation differed between microbial strains. For example, external casts caused by cell templating are produced by *R. palustris* (Fig. 1B, C), *M. thermophilus* (Fig. 1D) and *T. islandicus* (not shown). Conversely, cell casts on the surface of COA particles were not present for *C. aurantiacus*. Instead, the filamentous cells were incorporated within the interior of COA particles, as revealed by optical and fluorescence microscopy (Fig. 2A-C). Biomorphic cast features as well as rods or filaments encrusted with opal-A nanospheres were observed within natural COA from Strokkur, Iceland (Fig. 2D, E).

We observe characteristic spectral features for hydrated opal-A, as well as organic molecules (visible, FTIR, Raman), plus inorganic (Fe^{3+}, Fe-sulfides) and biomolecular (bacteriochlorophyll) signatures of microbial metabolism (Fig 3). FTIR analyses revealed aliphatic hydrocarbons (alkanes and alkenes), aromatic nitrogen-containing compounds and amides which were not observed in the sterile blank.

Discussion: Our experiments demonstrate that the cryogenic precipitation of opal-A can preserve morphological and geochemical evidence of microorganisms. Our results contrast with some previous investigations into microbial silicification at low temperatures [e.g., 6]. The precipitation of opal-A on and around microbes in these studies was characterised by the gradual growth of silica sheaths surrounding cells, and the formation of silica spheroids of up to 2 µm in diameter. However, in the current study, COA spheroids were small (~50 nm; Fig. 1), and cells were trapped, forming surface casts, or entirely encased during the freezing process. Thus, the cryogenic silicification of microorganisms differs from silicification under ambient (non-freezing) conditions where gradual nanoparticle growth and sinter sedimentation dominate [7].

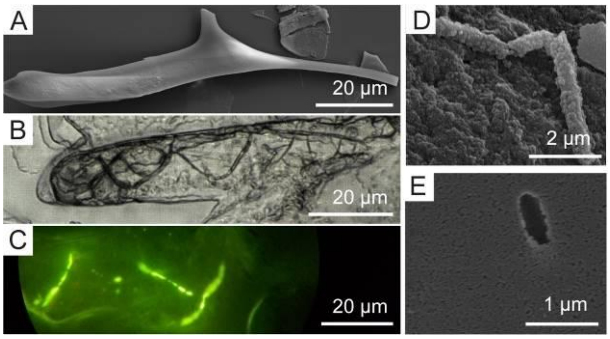

Fig. 2: *(A)-(C) COA particles containing cells of* C. aurantiacus. *Note the absence of cellular microfossils at the surface of the large COA particle in SEM image (A), whilst filaments are clearly visible within transparent COA particles under both optical (B) and fluorescence microscopy (C). (D) Possible microbial filament encrusted with opal-A microspheres from natural Strokkur COA. (E) Biomorphic casts on the surface of a natural COA particle from Strokkur.*

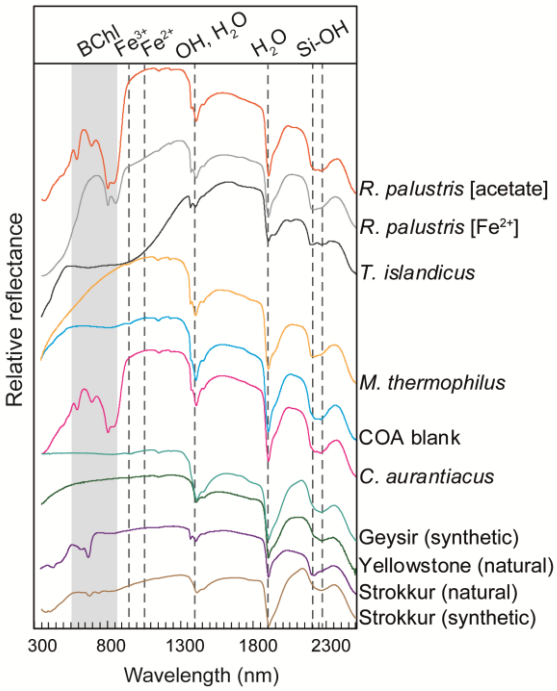

Fig. 3: *Visible – SWIR reflectance spectra showing absorption bands in the visible for bacteriochlorophyll pigments (BChl) and inorganic phases (dashed lines).*

The identification of biological compounds and inorganic compounds directly relating to microbial metabolism demonstrate the utility of combined FTIR, Raman and Vis-SWIR reflectance spectroscopy in the characterisation of such deposits on extraterrestrial surfaces such as Mars (e.g. MicrOmega, ISEM and RLS on ExoMars) and in future surface investigation of icy moons. We have shown that partitioning of microorganisms within ice-bound brine channels during cryogenic silicification can lead to the preservation of morphological and spectroscopic biosignatures.

References: [1] Ruff, S. W. *et al.* (2011) *JGR Planets* 116; [2] Hsu, H. W. *et al.* (2015) *Nature* 519, 207–210; [3] Waite, J. H. *et al.* (2017) *Science* 356, 155–159; [4] Channing, A. & Butler, I. B. (2007) *Earth Planet. Sci. Lett.* 257, 121–131 [5] Jones, B. & Renaut, R. W. (2010) *J. Sediment. Res.* 80, 17–35; [6] Westall, F. (1995) *Palaeontology* 38, 495–528. [8] Orange, F. O., (2013) *Astrobiology* 13, 163–176 (2013).