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### Geochemical and Metabolomic study of Yellowstone spring systems as Mars analogs

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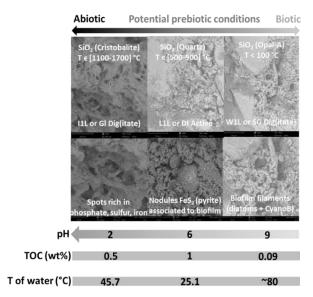
Yellowstone National Park (WY, USA) contains silicasaturated hot springs [1] with chemistry driven by subsurface (and pluvial) processes: minimum subsurface boiling hot springs fed by the deep hydrothermal source, or hot springs in regions where extensive subsurface boiling feeding springs that are predominantly residual liquid phase, predominantly condensed vapor phase, or a mixture of both [2]. These processes determine the geochemistry of the surface evaporates/precipitates collected for this study: i) minimum phase separation hot springs represented by alkaline (pH 8.7) at 'Similar Geyser' hot spring (W1L), ii) subsurface boiling residual liquid phase enriched in chloride and sulfate represented by pH 5.1 Dante's Inferno (L1L), and iii) subsurface boiling vapor phase rich in hydrogen sulfide (oxidizing to sulfuric acid) represented by pH 2.4 at 'Goldilocks' (I1L).

Each of these spring systems range water temperatures (25 – 80 °C) and elemental composition (e.g., Si, O, C, S, Al, Na, Fe, Ca, K, Mg, As, P, and N). These geochemical parameters impact the microbial community composition. pH drives microbial community composition, but does not seem to drive the mineral-organic, (a)gnostic (bio)signatures, or the sheltering of the organic matter (OM) content over time [2]. Metabolomic studies revealed a broader diversity of OM and distinct biomarkers from modern systems compared to a few thousand years old geological layers in the above spring systems (10% of OM left in the ancient system compared to the modern). We identified 122 with a flightlike derivatization-extracting process and pyrolysis-gas chromatograph coupled with a commercial mass spectrometer [3,4]. Liquid extraction with water or MeOH/DCM and SPME enabled the identification of 30 volatile analytes, including ketones, aldehydes, and carboxylic acids. Instruments under development (SCHAN and Orbitrap) for future spaceflight missions were used to detect additional bioindicators or biosignatures and enhance our metabolomic microbial community profiling reconstruction and microbial identification.

These biogeochemical and metabolomic studies allowed us to target and identify bioindicators and biosignatures relevant to Mars using these analog samples to describe what OM we may observe if life appeared on

Mars (similarly to Earth life [5,6]). Critically, identifying organic compounds associated with in situ microbial communities that can be preserved through early diagenesis is needed given the potential for alteration of OM [7]. With the MSL and Mars 2020 missions, we will likely analyze ancient hydrothermal systems similar to Yellowstone and perhaps with similar Early-Earth and Early-Mars chemistry [8,9]. Regardless of whether life emerged or evolved differently on Mars, the microscopic (coupled to geochemical/elementary/isotopic analysis presented in Figure 1) and mass spectroscopic analysis of Yellowstone samples (with agnostic and gnostic biosignatures) may be relevant to samples from Perseverance that are being returned to Earth in the frame of Mars Sample Return mission. Despite the absence of microscopic imaging data from Mars landers and rovers, Fig. 1 shows that a rich abundance of sulfur and/or phosphate and/or iron combined with bioindicators' or biosignatures' detection might ensure the robustness of potential past trace of life on Mars, and better inform sample selection for analysis and return moving forward.

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**Figure 1.** (A)gnostic signatures observed in these samples by scanning electron microscope and locally analyzed by energy dispersive X-ray spectroscopy, and completed by external isotopic and elementary analysis by an isotope ratio mass spectrometry.

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