DETERMINING ORIGIN OF ORGANIC MATTER IN MARTIAN SAMPLES USING METABOLOMICS

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Introduction: Central to the assessment of martian habitability is the availability of organic matter (OM) on the red planet. Complex OM has been detected both in martian meteorites [1] in the form of macromolecular carbon, and at Gale Crater by the SAM instrument [2]. The forthcoming generation of martian rovers is primed for OM detection, via the NASA Perseverance rover SHERLOC, and the ESA Rosalind Franklin rover RLS and MOMA instrument suites. With the prospect of Mars sample return getting ever closer [3], it is crucial that we develop ways to maximise the scientific return from the limited sample mass that will be available. The same is true for the small and precious martian samples we currently have on Earth – meteorites.

Here we demonstrate how the method of untargeted metabolomics in the form of liquid chromatography coupled with mass spectrometry (LC-MS) and rigorous bioinformatics analysis, is a sensitive and high-resolution technique capable of detecting trace amounts of OM in martian samples. Furthermore, we show that in some cases, terrestrial contamination can be beneficial - to constrain meteorite fall circumstances and fill in crucial blanks in meteorite terrestrial history.

The Lafayette Meteorite: Lafayette is one of 18 nakhlite meteorites, all thought to originate from the same site on Mars [4]. The fall history of the Lafayette meteorite is unconfirmed. Early reports state that it fell into the edge of a muddy pond in Tippecanoe County Indiana and was observed by a Purdue University student whilst fishing [5]. The student collected the stone and donated it to Purdue University sometime later. Initially it was misidentified by university staff as a glacial remnant and was curated accordingly [5]. In 1931 it was rediscovered in a drawer and identified by O. C. Farrington as a meteorite, with an intact fusion crust and clear flow features from atmospheric re-entry (which had previously led to its misidentification, as they resembled glacial striations). By this stage, the student could not be identified, and therefore the fall scenario could not be confirmed [5].

Methods: An interior sample of Lafayette, provided by the Natural History Museum of London, was powdered in a clean room environment at Durham University. Solvent soluble OM was extracted at SUERC by dissolving 3×30 mg samples of Lafayette in hexane, methanol, and dichloromethane, then pooling, and filtering. Triplicate blanks were introduced with every solvent and processed in an identical fashion alongside samples to identify laboratory and instrumental contamination. Alongside the Lafayette sample, extractions were also performed on triplicate 30 mg samples of JSC Mars1 (NASA martian soil simulant), as well as two terrestrial Mars analogues from Svalbard [6]. These analogue materials were used as a comparison for the OM detected. The full solvent extraction protocol is shown in Figure 1.

Once extracted, the samples were stored at -20 °C until untargeted metabolomics was carried out by performing hydrophilic interaction LC-MS at the Glasgow Polyomics facility using Dionex UltiMate

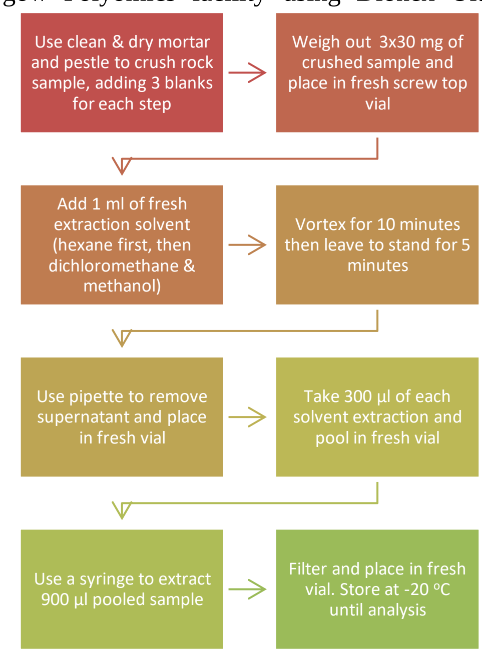

Fig. 1 Extraction protocol to dissolve solvent-soluble OM in meteoritic and analogue samples for LC-MS analysis

3000 RSLC system with a ZIC-pHILIC column.

Results: LC-MS data were initially processed as outlined in [7]. Metabolites (individual organic molecules detected by the MS) were identified by matching their exact mass to the extensive KEGG database of common metabolites [7]. Extracted ion chromatograms from individual metabolites were exported and converted to distribution graphs as outlined in [7]. Metaboanalyst software was used to

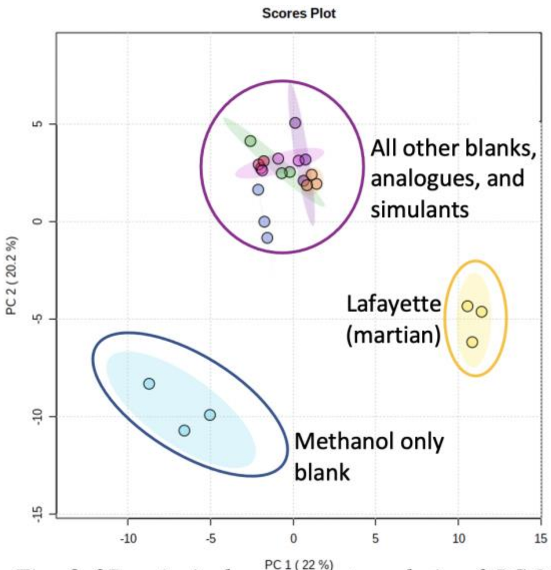

Fig. 2 2D principal component analysis of LC-MS data for detected peaks. Lafayette clusters separately, suggesting differences in the OM when compared to the other blanks, analogues and simulants. The methanol blank appears distinct as it contains no other solvent, thus did not dissolve all OM present.

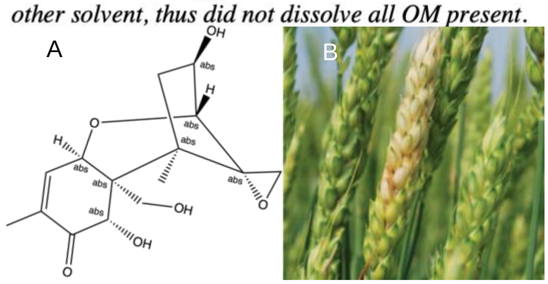

Fig. 2 A Structure of deoxynivalenol, a compound (commonly known as vomitoxin) detected in Lafayette and no blanks or analogues, using LC-MS. DON has an exact mass of 296.13. DON is a toxin produced by Fusarium Head Blight in wheat, causing premature bleaching in infected spikelets, as shown in B, adapted from [9].

carry out principal component analysis [8] (PCA) - see Figure 2. Any metabolites that were detected in both the blanks and Lafayette were ignored, and OM found only in Lafayette was further investigated.

Discussion: PCA carried out on LC-MS data highlighted that the OM detected in Lafayette was distinct from all other samples analysed (see Figure 2). Until compound-specific hydrogen isotope ratios are collected, we cannot distinguish for certain between martian and terrestrial OM within Lafayette, particularly as the fall history is unconfirmed. However, Lafayette has little terrestrial weathering and an intact fusion crust, suggesting the meteorite may be less contaminated than other meteoritic finds [5].

A metabolite putatively annotated as vomitoxin, otherwise known as Deoxynivalenol (DON), was detected in Lafayette samples, while it was below detection levels in the other sample groups. DON is a mycotoxin most commonly produced by the fungal pathogen *Fusarium graminearum*, one of the causative agents of Fusarium Head Blight (FHB). FHB is a disease which causes premature bleaching of wheat or corn [9], see Figure 3. DON is toxic to livestock and humans. Purdue University is in Indiana, a state dominated by agriculture, where FHB poses a semi-regular serious risk to livestock [9]. There are annual records of FHB occurrences at county level [10], meaning that the fall year of the meteorite can be potentially constrained to the years when FHB was prevalent in Tippecanoe County.

As outlined in [11], fusarium-infected plants generate elevated levels of the amino acids phenylalanine, tryptophan and tyrosine as part of their immune response to FHB. We did not detect levels of these amino acids above background levels, however initial data processing suggests detection of other metabolites in Lafayette only, that are putatively annotated as intermediates of the metabolic pathways of these three amino acids.

The mass of DON annotates several other compounds in LC-MS databases, so further work will be carried-out to confirm the identity of the detected peak. Targeted LC-MS experiments using a DON standard will then enable us to confirm whether DON is present in Lafayette samples, a hypothesis which is already supported by the prevalence of FHB in the region (Purdue has significant expertise in FHB as a result [10]). Repeats with a DON standard will confirm our fall-scenario-constraining dataset.

Nininger's account of Lafayette's history states that an African American student found the stone [5]. There were very few African American Purdue students in the early 20th Century. We are working with the University Library team hoping to cross-reference departmental attendance records with archives of FHB prevalence in Tippecanoe County to determine the most likely year the meteorite fell and ultimately identify the student and contact any living relatives.

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