Textural and Mineralogical Characteristics of Microbial Fossils Associated with Modern and Ancient Iron (Oxyhydr)Oxides: Terrestrial Analogue for Sediments in Gale Crater

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Abstract

Iron (oxyhydr)oxide microbial mats in modern to ~100 ka tufa terraces are present in a cold spring system along Ten Mile Graben, southeastern Utah, USA. Mats exhibit morphological, chemical, and textural biosignatures and show diagenetic changes that occur over millennial scales. The Jurassic Brushy Basin Member of the Morrison Formation in the Four Corners region of the USA also exhibits comparable microbial fossils and iron (oxyhydr)oxide biosignatures in the lacustrine unit.

Both the modern spring system and Brushy Basin Member represent alkaline, saline, groundwater-fed systems and preserve diatoms and other similar algal forms with cellular elaboration. Two distinct suites of elements (1. C, Fe, As and 2. C, S, Se, P) are associated with microbial fossils in modern and ancient iron (oxyhydr)oxides and may be potential markers for biosignatures. The presence of ferrihydrite in ~100 ka fossil microbial mats and Jurassic rocks suggests that this thermodynamically unstable mineral may also be a potential biomarker.

One of the most extensive sedimentary records on Mars is exposed in Gale Crater and consists of non-acidic clays and sulfates possibly of lacustrine origin. These terrestrial iron (oxyhydr)oxide examples are a valuable analogue because of similar iron- and clay-rich host rock compositions and will help (1) understand diagenetic processes in a non-acidic, saline lacustrine environment such as the sedimentary rocks in Gale Crater, (2) document specific biomediated textures, (3) demonstrate how biomediated textures might persist or respond to diagenesis over time, and (4) provide a ground truth library of textures to explore and compare in extraterrestrial iron (oxyhydr)oxides, where future explorations hope to detect past evidence of life. Key Words: Biogeochemistry—Mars—Biosignatures—Diagenesis—Iron oxides. *Astrobiology* 14, 1–14.

1. Introduction

1.1. Background

BIOSIGNATURES ARE preserved fingerprints of life interacting with fluid and rock, particularly where microbes enhance mineral precipitation during deposition and/or early diagenesis (Farmer and Des Marais, 1999; Banfield *et al.*, 2001; Cady, 2001; Cady *et al.*, 2003; Westall, 2008; Schopf *et al.*, 2010). Three types of biosignatures are described, as follows: (1) morphological microfossils that retain the carbonaceous remains of cells, (2) microbially influenced sedimentary structures such as stromatolites and chemofossils that include biominerals, and (3) isotopic signatures or concentrations of trace elements specific to sequestration by microbes (Cady *et al.*, 2003; Westall, 2008).

Establishing biogenicity of putative morphological microbial fossils can often be challenging (Farmer and Des Marais, 1999; Cady *et al.*, 2003; Schopf *et al.*, 2010). The following criteria have been proposed to show biogenicity (Cady, 2001):

- (1) Features must occur in a thin section in order to show they are not modern contaminants.
- (2) The features must occur in sedimentary or low-grade metamorphic rocks.
- (3) Microfossils must be larger than the smallest known extant organisms (~0.01 μm).
- (4) The features must be composed of kerogen to show they contain organic carbon.
- (5) The features must occur with others of similar morphology.

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- (6) The features must be hollow to show a biotic structure.
- (7) The features must show cellular elaboration.

If the features do not meet, or only meet, criteria 1–4, they are pseudofossils (features that appear to be fossils but cannot be confirmed). Possible fossils meet criteria 1–5, and probable fossils meet six of the seven criteria (Cady, 2001).

Preservation of biosignatures occurs by many different methods and in many different settings, such as tufas, clay-rich shales, water-laid pyroclastics, and some iron formations (Allison and Briggs, 1991; Farmer and Des Marais, 1999; Cady, 2001; Campbell *et al.*, 2004; Kolo *et al.*, 2009; Rainey and Jones, 2010). Permineralization by iron preserves cell structure during early diagenesis (Parenteau and Cady, 2010), and three-dimensional preservation of microbial fossils also occurs in concretions (Berner, 1968; Allison and Pye, 1994). Shallow evaporative, high-salinity environments are particularly well suited for preservation of microbial fossils (Knoll, 1984; Vogel *et al.*, 2010). However, preservation of any type of microbial fossil or chemical/textural biosignature depends on the degree of alteration during diagenesis and factors such as exposure to diagenetic fluids (Cady, 2001; Westall, 2008).

Iron (oxyhydr)oxide minerals have an outstanding capacity to preserve biogenetic fingerprints—both by permineralization of cell structure (Cady and Farmer, 1996; Konhauser *et al.*, 2003; Rancourt *et al.*, 2005; Westall, 2008; Parenteau and Cady, 2010) and by preserving diagnostic trace element configurations and/or kinetically unusual mineral phases (Westall, 2008; Chan *et al.*, 2009). In this respect, the microbial mats at the Ten Mile Graben spring system and the Brushy Basin Member both have excellent biosignature preservation potential due to their iron-rich composition.

1.2. Purpose of study

The purpose of this study was to compare modern iron (oxyhydr)oxide microbial precipitates in tufas (cold-water carbonate precipitates) at a cold springs along Ten Mile Graben in southeastern Utah with ~100 ka tufa examples from the same spring to determine diagenetic changes over millennial scales. Diagnostic morphological and chemical similarities in iron (oxyhydr)oxides were documented and then compared to similar features in the Jurassic Brushy Basin Member of the Morrison Formation to discern ancient biosignatures also preserved in iron (oxyhydr)oxides. Despite different depositional settings, both of these formations are alkaline, saline, groundwater-fed systems. The modern spring system provides a starting point to recognize biosignatures preserved in iron (oxyhydr)oxides because of the documented presence of microbial mats (Stilton *et al.*, 2004; Burnside, 2010), as well as a published age for ancient tufas from the same spring system (Burnside, 2010).

The Brushy Basin Member preserves distinctive alkaline saline Lake T'oo'dichi' sediments and was chosen for this study because of the hypersaline geochemistry and alkaline early diagenetic mineral alteration patterns (Turner and Fishman, 1991). Although so-called “extremophiles”—such as halophiles—likely inhabited the water and subaqueous sediments, abundant bioturbation features (*e.g.*, charophyte molds, root traces, burrows, and dinosaur bones)

suggest that macroscopic biota was common in interbeds where paleosols developed and/or when water chemistries were circumneutral (Turner and Peterson, 2004).

1.3. Applications to Mars

One of the most extensive sedimentary stratigraphic sections (~5 km thick) on Mars is exposed in Mount Sharp at Gale Crater (Milliken *et al.*, 2010; Thomson *et al.*, 2011). The unit is composed, in part, of clays that likely formed in a non-acidic, lacustrine environment (Cabrol *et al.*, 1999; Milliken *et al.*, 2010; Thomson *et al.*, 2011; Agle and Brown, 2013; Stack *et al.*, 2013). The Brushy Basin Member may provide an excellent terrestrial analogue for understanding lithological, diagenetic, and biogeochemical histories of these similar iron- and clay-rich shales on Mars. However, the terrestrial example contains abundant macroscopic biotic features, and microbes almost certainly caused the decay of these larger organisms; decay of plants and animals is not expected to have occurred on Mars. Nevertheless, certain facies (vuggy clay-/siltstones) in the terrestrial example exhibit restricted biodiversity that is limited to charophytes (microscopic algae; Turner and Peterson, 2004; Potter-McIntyre, 2013). A similar vuggy, fine-grained facies has recently been documented at Gale Crater (Stack *et al.*, 2013), and if life ever existed on Mars, it was likely also very restricted in terms of biodiversity. The study of the Brushy Basin Member presented here examines microbial evidence solely in these charophyte molds (vugs)—the limited-biodiversity facies—to provide a good analogue to the rocks at Gale Crater.

The study of extremophiles is of increasing importance in the search for extraterrestrial life (*e.g.*, Rothschild and Mancinelli, 2000) because any life that existed on Mars would be, or would have been, extreme compared to Earth standards. These terrestrial iron (oxyhydr)oxide examples (from Ten Mile Graben spring system and the Brushy Basin Member) will (1) document specific biomediated textures and mineral phases from a water-deposited, alkaline saline analog unit, (2) show how these textures and minerals might persist or respond to diagenesis over time, and (3) provide a ground truth library of textural and mineralogical biogenic fingerprints to explore and compare in extraterrestrial iron oxides.

2. Study Area

2.1. Ten Mile Graben

A CO₂- and hydrocarbon-charged cold spring system consisting of three major springs, Crystal Geyser, Big Bubbling Spring, and Little Bubbling Spring, emanates along Ten Mile Graben fault system south of Green River, Utah (Fig. 1). This system was chosen for study because modern microbial precipitates of aragonite and iron (oxyhydr)oxide are present in the water around the spring source (Stilton *et al.*, 2004; Burnside, 2010). The published pH of the water from this spring system is ~6.55 (Stilton *et al.*, 2004). Water is supersaturated with respect to calcite; thus aragonite precipitates readily (the disordered CaCO₃ precipitates because it is kinetically favored even though it is thermodynamically unstable). It is possible that the co-precipitation of both the aragonite and the iron

FIG. 1. Study areas. (A) Lake T'oo'dichi' centered on the Four Corners region of the western USA. Bull's-eye pattern is clay mineral zones with increasing alteration basinward (S=smectite; C=clinoptilolite; An=analcite; Al=albite). Study sites are marked in yellow (TM=Ten Mile Graben; FPA=Fruita Paleo Area; BH=Blue Hills; RG=Rattlesnake Gulch; MZ=Montezuma Creek; MC=McElmo Canyon). (B) Big Bubbling Spring in Ten Mile Graben. (C) Sample site in partially lithified microbial mat near the source of Big Bubbling Spring; unlithified sample was collected directly from vent. Scale bar is 15 cm long. (D) Brushy Basin Member outcrop in Montezuma Creek exhibiting variegated coloration. Outcrop shown in photo is ~15 m. (E) Terraces of tufa deposits (~100 ka) at Crystal Geyser in Ten Mile Graben. Terraces are 3–5 m high. (Color graphics available online at www.liebertonline.com/ast)

(oxyhydr)oxide is due to microbial interaction because these minerals precipitate under different pH conditions and because they are both metastable. Additional evidence for microbial mediation of the iron (oxyhydr)oxide is the extremely low concentrations of iron in the spring water (ranging from undetectable to 0.14 mmol/L). Because the water is undersaturated with respect to iron (oxyhydr)oxide, a simple abiotic oxidation reaction would likely be insufficient to produce the oxidized iron minerals that turn these mats red.

Ancient tufas from the same system form terraces along the fault system that are <400 ka, established by U-Th dating (Burnside, 2010); therefore, it is possible to compare modern samples with diagenetically altered (and accurately

dated) ancient examples of microbially precipitated iron (oxyhydr)oxides. For this study, we use modern examples from Big Bubbling Spring and Little Bubbling Spring and samples from an ancient ~100 ka tufa terrace at Crystal Geyser (Fig. 1). The remote modern spring sample locations were chosen to limit anthropogenic interference with microbial mats.

2.2. Morrison Formation, Brushy Basin Member

The Jurassic Brushy Basin Member of the Morrison Formation is a variegated shale formation that extends across the Colorado Plateau in the western USA. The Morrison Formation is renowned for its dinosaur remains,

TABLE 1. OBSERVED BIOSIGNATURE FEATURES, DESCRIPTIONS, AND CRITERIA SUPPORTING BIOGENICITY

Description	Samples	Analytical Method	1. Thin Section/ Rock	2. Sed. host rock	3. Size	4. Carbon	5. Others w/ Similar Morphology?	6. Hollow?	7. Cellular Elaboration?	Biogenic?
Segmented stalks coated w/ FeO	Modern and ancient tufas	Petrography	Yes	Yes	0.2-0.3 mm	Yes	Yes	Not observed	Yes	Probable
Branching filaments with equal width	Modern tufas, Jmb	SEM	Yes	Yes	1µm wide	?	Yes	Not observed	Not observed	Possible
Framboidal FeO habit	Modern tufas	SEM	Yes	Yes	10µm	?	Yes	No	N/A	Possible
Siliceous, fenestral tests	Modern unlithified sediments, Jmb	SEM	Yes	Yes	~4µm wide	?	Yes	No	Not observed	Probable
Spheroidal FeO along edges of vugs; associated with diatoms	Jmb (thin sections, hand samples)	SEM	Yes	Yes	~5µm diam.	Yes	Yes	No	N/A	Probable
Rectangular algal forms with internal cellular structures (plastids)	Jmb (HF dissolved sample)	SEM	No	Yes	~10 by 25µm	?	Yes	Yes	Yes	Possible
Oval form with radiating segments and central globular structure	Jmb (HF dissolved sample)	SEM	No	Yes	20 by 25µm	?	Yes	Yes	Yes	Possible

particularly the preservation of large sauropods (*e.g.*, Chure, 1994; Evans and Chure, 1998; Henrici, 1998). The entire Morrison Formation was deposited over a period of 7 million years from 155–148 Ma (Kowallis *et al.*, 1998; Turner and Peterson, 2004). The Brushy Basin Member is capable of capturing paleobiological information because it is a clay-rich shale—one of the most optimal environments for preserving microbial fossils (Farmer and Des Marais, 1999).

The upper part of the Brushy Basin Member was deposited in a groundwater-fed, ephemeral, alkaline saline lake system named Lake T'oo'dichi' that was centered on the Four Corners region (Turner and Fishman, 1991; Fig. 1). A back arc basin existed to the west, and associated volcanics supplied copious amounts of ash to the depositional basin (Turner and Fishman, 1991; Turner and Peterson, 2004). Clay mineralogy has been extensively documented, and clay mineral zones (present in tuffs) form a bull's-eye pattern that shows increasing early diagenetic alteration to the basin center (Turner and Fishman, 1991; Fig. 1). The clay mineral zones are—from margin to center—smectite, clinoptilolite, analcime, and albite. The Morrison Formation is well known for preserving extensive flood plain, lacustrine, and fluvial deposits and has been extensively studied for the rich paleoecological information it preserves about the late Jurassic. The stratigraphic context is so well documented, it

allows for more-detailed, pore- to outcrop-scale diagenetic analysis presented in this study.

3. Methods

Partially lithified samples about 10 cm thick were collected with a shovel from Big Bubbling Spring and Little Bubbling Spring (Fig. 1). An unlithified sample was collected from the vent at Big Bubbling Spring. Lithified samples from an ~100 ka tufa at Crystal Geyser were selected for the presence of preserved iron (oxyhydr)oxide bands (Fig. 1). Outcrops of Brushy Basin Member ($n=6$) were selected for exposure and location from Lake T'oo'dichi' basin center to margin (Fig. 1). Representative samples of concretions ($n=42$) and host rock ($n=66$) were collected from six locations for mineralogical, petrographic, and chemical analysis.

Polished thin sections were prepared and examined (modern tufas, $n=5$; ancient tufas, $n=1$; Brushy Basin Member, $n=53$) to identify possible biogenic textural features and to select a smaller number of appropriate samples ($n=9$) and sites for further analyses. Due to the presence of abundant microbial features in the modern spring samples, large sample numbers were not needed. High-resolution images were made of selected spots on one ancient tufa

FIG. 2. Photomicrographs (plane light) of modern (A, B) and ancient (C, D) tufas with iron (oxyhydr)oxides from Ten Mile Graben. (A) and (B) Big Bubbling Spring partially lithified sample exhibiting segmented algal stalks encrusted with iron (oxyhydr)oxides. (C) and (D) Ancient tufa from near Crystal Geyser exhibiting similar features but with a higher degree of crystallinity than the modern example. (Color graphics available online at www.liebertonline.com/ast)

sample and three Brushy Basin Member samples with an FEI NanoNova scanning electron microscope (SEM).

Representative samples of the Brushy Basin Member ($n=4$) were selected for HF (hydrofluoric acid) dissolution because of the presence of possible microbial precipitates in obvious burrows or ~ 1 by 3 mm vugs (interpreted as small burrows or charophyte molds; Turner and Peterson, 2004). Bioturbated pieces of samples (<0.25 g) were disaggregated and dissolved in hydrofluoric acid. The slurry was decanted, rinsed with water, and allowed to air-dry on microscope slides. Slides of the slurry were examined with reflected light microscope and SEM to detect the presence of cells or other carbonaceous material. The slurry technique was chosen over an *in situ* detection technique (*i.e.*, Raman spectroscopy) because the fluorescence of the materials overwhelmed the Raman spectra despite experimentation with multiple techniques and wavelengths that rendered this an unsuitable analytical technique for the Brushy Basin Member samples.

X-ray diffraction (XRD) spectra allowed determination of mineralogy. Modern tufa samples ($n=4$) were air-dried and powdered, then analyzed from $2-65^\circ 2\theta$. Powdered samples from the Brushy Basin Member ($n=29$) were also analyzed

from $2-65^\circ 2\theta$. Quantitative Elemental Mineralogy using SCANNing electron microscope (QEMSCAN) produces mineralogical images of entire thin sections ($n=6$) at $10\ \mu\text{m}$ pixel spacing.

Whole rock analysis, performed by ALS Chemex in Reno, Nevada, allowed identification of trace element concentrations and was also used to select target elements for further analyses. One sample from Little Bubbling Spring and four samples from Big Bubbling Spring were analyzed. A total of 55 samples from the Brushy Basin Member were selected for analysis.

Laser ablation induced coupled mass spectroscopy (LA-ICP-MS) produced spectra to identify main and trace elemental configurations across possible biological targets. Transects of $1600\ \mu\text{m}$ were made across possible biological features and possible biomediated mineral structures (*e.g.*, spheres) identified with petrography. Targets ($n=8$) on two modern tufa samples and targets ($n=3$) on one ancient tufa sample were chosen along with targets ($n=19$) on four Brushy Basin Member samples. Trace element choices were identified with whole rock analysis. Principal component analysis (PCA) was used to identify correlations between elements.

FIG. 3. SEM images of morphological microbial fossils; left column is modern samples from Big Bubbling Spring (BB), and right column is comparative Brushy Basin Member samples (Jmb). (A) and (B) Filaments in modern and ancient samples. Arrows point to branching morphology. (C) Modern iron (oxyhydr)oxide framboid, platy minerals that encircle the framboid, and spatially associated twinned pseudo-hexagonal aragonite crystals. (D) Ancient diatom tests and iron (oxyhydr)oxide spheroid. (E) Modern diatom test. (F) Ancient diatom tests and twinned mineral.

4. Results

4.1. Ten Mile Graben samples

Iron-rich mats preserved in modern Big and Little Bubbling Spring and ancient Crystal Geyser tufas show similar segmented stalk morphologies in petrographic microscopy (Table 1; Fig. 2); however, some early diagenetic alteration is observed in the ancient example (Fig. 2C, 2D). In the modern mat, segmented stalks consist of slightly rounded segments <1 mm in length. The stalks and surrounding iron (oxyhydr)oxides are diffuse and fairly amorphous. In the ancient tufa sample, stalks appear more rigid, and the exterior is well defined; however, the segmented morphology is still apparent.

Modern microbial mats also exhibit other features [branching filaments and iron (oxyhydr)oxide framboids] and diatom fossils visible with SEM (Fig. 3). Filaments are uniform in diameter, and individual stalks range from 0.1 to 1 μm in width. These filaments are slightly curved and exhibit branching (Fig. 3A). Iron (oxyhydr)oxide framboids (approximately 10 μm in diameter) were identified (Table 1; Fig. 3C). Framboids exhibit a botryoidal surface texture (individual globules are <0.1 μm in diameter; Fig. 3C). Framboids are encircled by platy minerals that are $\sim 0.5 \mu\text{m}$ thick. Additionally, framboids are spatially associated with clusters of columnar, pseudohexagonal twins of aragonite (identified via SEM and XRD). In unlithified sediment collected from the vent of the modern spring, diatom tests are present (Table 1; Fig. 3E).

In the modern tufa samples ($n=3$), the main minerals identified with XRD are aragonite and calcite; quartz, halite, hematite, 6-line ferrihydrite, and illite/smectite are also present. In the unlithified sample from the spring vent, the

minerals present are aragonite, calcite, 6-line ferrihydrite, and quartz.

4.2. Brushy Basin Member samples

The Brushy Basin Member has many macroscopic biogenic features such as charophyte molds (vugs), burrows, petrified wood, and dinosaur bones (Fig. 4). With petrographic microscopy, examination of charophyte molds can provide clues with which to begin the search for microscopic biogenic features and associated biogenic mineral textures. In the present study, petrographic analysis indicated that elongate charophyte molds (~ 1 mm wide by 3 mm long) are typically filled with massive calcite or albite (Fig. 5). Chlorite is also commonly present in charophyte molds and exhibits lath and/or radiating acicular habits that overprint the massive calcite/albite fill. Laths typically radiate inward from the edges of charophyte molds, and radiating acicular habits typically are in circular patterns in the centers. Edges of charophyte molds commonly have reduction halos (Fig. 5), and iron (oxyhydr)oxide spheroids are clustered along the edges in some examples (Table 1).

Features observed with SEM in the Brushy Basin Member included filamentous structures, spheroids, and diatom fossils (Table 1; Fig. 3). Branching morphology is present in the filamentous structures, and the filaments are of uniform width (ranging from 0.1 to 1 μm in width for individual filaments; Fig. 3B). Although it is possible that the filamentous structure observed in the Brushy Basin Member is a modern contaminant, this is not likely, because the observation was made on a polished thin section that was taken from a fresh surface of a sample (Cady, 2001; Westall, 2008). Diatom tests are present along the interior edge of a

FIG. 4. Macroscopic biosignatures (trace fossils and permineralized bones) in the Jurassic Brushy Basin Member. (A) Vugs (interpreted as charophyte molds or larvae hatches) and burrows. (B) Reduction halos around root traces. (C) Highly burrowed horizon. (D) Fossilized dinosaur femur; scale is 12 cm. (Color graphics available online at www.liebertonline.com/ast)

FIG. 5. Petrographic examples of authigenic minerals in Brushy Basin Member samples. (A) Albite and chlorite-filled charophyte mold. (B) Inset of view (A). (C) Spheroidal iron (oxyhydr)oxides along edge of charophyte mold. (D) Inset of view (C). Color images available online at www.liebertpub.com/ast

FIG. 6. SEM images showing cellular elaboration in sample MZ4 dissolved in HF. (A) Rectangular alga and (B) inset showing internal structure. (C) Rectangular alga and (D) inset showing internal structure (likely plastids). (E) Circular alga and (F) inset showing internal structure similar to internal structure of views (C) and (D).

charophyte mold directly abutting an unidentified twinned mineral (possibly hematite or goethite; Cornell and Schwertmann, 2003) in one example and spheroids of iron (oxyhydr)oxide in another example (Fig. 3D, 3F). Slurry from charophyte molds in Brushy Basin Member samples ($n=4$) dissolved in HF and examined with SEM show rectangular structures ($\sim 30 \mu\text{m}$ long by $10 \mu\text{m}$ wide) and oval structures ($\sim 30 \mu\text{m}$ on the long axis). These structures exhibit internal structures that are oval and rectangular and $0.5 \mu\text{m}$ long (Table 1; Fig. 6).

The Brushy Basin Member samples analyzed with XRD ($n=29$ from all clay mineral zones) show that clays are dominated by smectite, but illite, illite/smectite, clinoptilolite, chlorite, analcime, and kaolinite are also present. Iron minerals are dominated by goethite and hematite, but 6-line ferrihydrite and siderite are also present. Iron phosphate, apatite, and calcite are common. The clay minerals in samples do not typically correlate with the clay mineral zones of Lake T'oo'dichi' because those diagnostic minerals are present only in tuffs and not in the more common siltstone/claystone facies analyzed in this study (Turner and Fishman, 1991).

Area percent mineralogy reported by QEMSCAN shows detrital minerals such as quartz, feldspars, and biotite. Authigenic minerals include albite, barite, calcite, chlorite, clinoptilolite, analcime, apatite, and iron (oxyhydr)oxides. Porosity ranges from 17% in a sandstone sample (RG1) to 0.3% in a claystone sample (MZ3). Trace amounts of organic matter were detected (0.01–0.02 area %). Organic carbon classification with QEMSCAN is based on brightness value as well as unusually high counts of carbon. In sample MZ3, charophyte molds are filled with barite, calcite, chlorite, and albite (Fig. 7). Iron (oxyhydr)oxide is present throughout the sample as well as in charophyte molds. Small amounts of organic matter are also present throughout the sample (Fig. 7).

4.3. Principal component analysis and correlation of trace elements

Principal component analyses were compiled for LA-ICP-MS transects across modern tufa samples ($n_{\text{transects}}=8$), an ancient tufa sample ($n_{\text{transects}}=3$), and four Brushy Basin Member samples ($n_{\text{transects}}=8$). In four transects across two modern tufa samples, carbon correlates with iron (Table 2). Carbon correlates with iron in three transects across one ancient tufa sample as well as in a spheroidal iron (oxyhydr)oxide precipitate inside a charophyte mold in a Brushy Basin Member sample (MZ29; Fig. 8, Table 2). Arsenic is also typically correlated with carbon and iron in these samples.

In one transect in a modern tufa sample, carbon correlates with sulfur and selenium. These elements also correlate in five transects across three possible biogenic features in three Brushy Basin Member samples (MZ3, MZ4, and EC2). Phosphorous typically correlates with sulfur and selenium as well (Table 2).

A third group of anomalous correlations with carbon is present (Table 2). One transect across one modern tufa sample and one transect across one Brushy Basin Member sample show a correlation between carbon and sodium. One transect across one modern tufa sample and one transect

FIG. 7. QEMSCAN image of charophyte molds and organic carbon in Brushy Basin Member. (A) Image of sample MZ3 at $5 \mu\text{m}$ pixel spacing. Square shows area of views (B) and (C). (B) Close-up of square in (A). (C) Image showing only organic carbon pixels (red) overlain on view (B). (Color graphics available online at www.liebertonline.com/ast)

across one Brushy Basin Member sample show carbon correlated with manganese. A transect across one modern tufa sample shows carbon correlated with copper. In three out of these five transects, silicon also correlates with carbon.

In summary, features in modern and ancient tufas are similar, although some recrystallization is observed in the ancient examples. Diatoms and segmented stalks are present in the tufa mats. Charophyte molds in Brushy Basin Member samples exhibit mineral fill with lath and acicular chlorite habits as well as spheroidal iron (oxyhydr)oxide habits along the edges. Fossil diatoms are spatially associated with the spheroidal iron (oxyhydr)oxide mineral habits. QEMSCAN showed small amounts of organic carbon associated with the charophyte molds, and 6-line ferrihydrite was detected with XRD in modern and ancient samples. Two distinct elemental suites are associated with microbial features in both the modern and ancient tufas and the Brushy Basin Member samples: 1. C, Fe, As and 2. C, S, Se, P (Table 2).

5. Interpretation and Discussion

5.1. Interpretation of modern and ancient tufas and Brushy Basin Member features

Iron-rich mats contain stalks interpreted as algal fossils because of the uniform diameters and segmentation in both the modern and ancient tufas (Table 1, Fig. 2). Some

TABLE 2. THREE GROUPS OF ELEMENTAL CORRELATIONS FROM PCA OF LA-ICP-MS TRANSECTS

C, Fe Correlation		C, S, Se Correlation		Anomalous		
Sample #	Other Corr. Elements	Sample #	Other Corr. Elements	Sample #	Elements Corr. w/ C	Other Corr. Elements
LB1 MSD1	none	BB2 MSD 3	none	BB2 MSD1	Na	none
BB2 MSD2	Cu, Na, Ni, Al	MZ4 1	As, Ni, P, U	LB1 MSD2	Mn	none
BB2 MSD4	As, Ba, Ca, Mn, Na, P, S, Sr, U	MZ3 1	P, U	LB1 MSD3	Cu	Ca, Ni, Si
BB2 MSD5	As, Ba, Mn	EC2 1	none	MZ3 2	Mn, Si	As, Cu, Ni, S
CG1 AT1	As, Na, Si, U	EC2 3	As, Ba, P	MZ29 3	Al, Na	Si
CG1 AT2	As, Na, Mn, U	EC2 4	none			
CG1 AT3	Mn, Ni, S					
MZ29 1	As, Cu, Na, Ni					

Tufa samples are in red font and Jmb samples are in blue font.
(Color graphics available online at www.liebertonline.com/ast)

degradation was observed in the ancient examples (Fig. 2C, 2D). In the modern partially lithified mats, segmented algal stalks exhibit well-developed segments with amorphous iron (oxyhydr)oxides precipitated on and around them (Fig. 2A, 2B). In the ancient tufa samples, the segmented algal fossils are present, although they are smaller than the modern features and the iron (oxyhydr)oxides appear to have recrystallized to larger crystals (less amorphous) via Ostwald ripening (Lifshitz and Slyozov, 1961; Ortoleva 1982; Sultan and Ortoleva 1993; Müller and Ross 2003; Fig. 2C, 2D). High-resolution SEM analysis of modern tufas showed fibrous structures that were interpreted as microbial features due to the uniformity of diameters and branching morphologies (Fig. 3A). These are spatially associated with framboidal structures that are hypothesized biomediated iron (oxyhydr)oxide habits [and likely iron (oxyhydr)oxide pseudomorphs after pyrite precipitated by sulfur-reducing bacteria; Table 1, Fig. 3C; *e.g.*, Popa *et al.*, 2004; Cavalazzi *et al.*, 2012]. Diatoms are present in the unlithified sample from the spring vent (Table 1, Fig. 3E).

In the Brushy Basin Member, charophyte molds (vugs) provide a starting point to investigate microscopic bio-signatures. These features formed in a lake bed, and charophytes are microscopic algae, so these vugs provide an excellent starting place to look for microbial involvement with the mineralization process. Suspected paleomicrobial features in charophyte molds are spheroidal iron (oxyhydr)oxide precipitates along the edges and reduction halos (Fig. 5). SEM images show branching filaments similar in morphology to modern tufas (Table 1, Fig. 2B). Degraded diatoms present in close proximity to iron (oxyhydr)oxide spheroids lend support for the hypothesis that spheroids may be a biomediated mineral habit (Cavalazzi *et al.*, 2012;

Table 1, Fig. 3D). Dissolution in HF of the siliceous portion of the rocks with charophyte molds revealed many rectangular and circular features with internal structures that were interpreted as algal fossils with cellular elaboration (Table 1, Fig. 6).

5.2. Biogenicity of Jurassic Brushy Basin Member features

The first requirement for proving biogenicity of putative microbial fossils is that features must occur in a thin section (in order to show they are not modern contaminants; Cady, 2001; Westall, 2008). Most of these microbial features—such as diatoms and suspected biomineral habits—do occur in thin section; however, careful dissolution of the charophyte molds reveals other types of algal microbial fossils (Table 1, Fig. 6). Although it is possible that algal microbial features present in the slurry are modern contaminants, diatoms present in thin section suggest that these other forms of algae associated with the diatoms are likely fossil algae as well.

The second requirement is that microbial features must occur in sedimentary or low-grade metamorphic rocks (Cady, 2001), and the Brushy Basin Member is a lacustrine volcanoclastic mudrock (Turner and Fishman, 1991). The third requirement is that microfossils must be larger than the smallest known extant organisms ($\sim 0.01 \mu\text{m}$; Cady, 2001). The features in the Brushy Basin Member are all $\geq 10 \mu\text{m}$.

The fourth requirement of biogenicity is that features must be composed of kerogen (Cady, 2001). We were unable to detect kerogen with Raman spectroscopy because, despite experimentation with multiple wavelengths and types of Raman spectroscopy, fluorescence overwhelms the

FIG. 8. LA-ICP-MS data for modern (A) and ancient (B) tufas and Brushy Basin Member sample (C). Blue dots are actual counts, and dark brown line shows a five-period moving average. Pink lines on photomicrographs show transect for laser ablation and are all $\sim 1600 \mu\text{m}$ in length. (Color graphics available on-line at www.liebertonline.com/ast)

signal on most samples. Organic phosphorous was detected with Raman spectroscopy in one sample that was collected directly above a fossilized dinosaur bone. Carbonaceous material was visible with reflected light microscope in slurry from all dissolved samples ($n=3$). Carbon signals from LA-ICP-MS increase over the putative forms as would be expected if they were carbonaceous, and minor amounts of organic carbon were detected with QEMSCAN. Although kerogen was not directly detected, the combination of multiple lines of evidence suggests that these features are likely carbonaceous.

The fifth requirement is that multiple features with similar morphology are present and the feature is not an isolated, lone form (Cady, 2001). Diatoms, twisted stalks, filaments, and rectangular and circular algal forms with internal structures are quite common in the samples examined (Table 1, Figs. 3, 5, 6). Clusters of spheroids are present in macroscopic biogenic features (charophyte molds), and spheroids are spatially associated with diatom fossils (Figs. 3C, 3D, 5). This supports the hypothesis that spheroidal mineral habits may be biomediated (Banfield *et al.*, 2001; Table 1).

The sixth criterion is that the features must be hollow, and some algal examples show hollow interiors imaged with SEM (Table 1, Fig. 6). Lastly, the seventh requirement is that features must show cellular elaboration. All the algal features, such as the diatoms and the rectangular and oval algal forms, show cellular details (Table 1, Figs. 3, 6). These features completely meet six of the seven criteria for proving biogenicity, and though kerogen was not detected, these features are likely carbonaceous. Thus, these features are classified as probable microfossils (Table 1).

5.3. Mineralogical and chemical biosignatures

Mineralogy in the modern (unlithified and lithified) and ancient tufas are similar and contain disordered, kinetically favored mineral phases such as 6-line ferrihydrite and aragonite. In the modern unlithified sample, only disordered phases (6-line ferrihydrite and aragonite) are present. Finally, some of the Jurassic Brushy Basin Member samples contain 6-line ferrihydrite. In lab bench experiments, ferrihydrite is very unstable and recrystallizes to more stable phases over the course of days or weeks (Cornell and

Schwertmann, 1996; Kukkadapu, 2003). However, organic polymers retard the recrystallization process (Chan *et al.*, 2009); therefore, the presence of 6-line ferrihydrite in lithified tufas and ancient rocks can function as a potential biosignature indicator (Châtellier and Fortin, 2004; Kennedy *et al.*, 2004; Schwertmann *et al.*, 2005; Chan *et al.*, 2009). It is possible that the ferrihydrite in the Jurassic samples is a weathering product, but trenches were dug to obtain fresh samples and avoid contamination by surface weathering. Thus, the presence of 6-line ferrihydrite is most likely an authigenic mineral precipitate preserved due to biomediation.

Microbial fossils along the edges of charophyte molds show evidence of microbial mediation of minerals. The close spatial association of unusual crystal habits (spheroids) with algal fossils, such as diatoms, suggests that these mineral habits formed via biomediation because crystal growth in the presence of organic ligands can alter the stability of crystallographic surfaces (Mann *et al.*, 1993; Albeck *et al.*, 1996; Banfield *et al.*, 2001; Chan *et al.*, 2009). Spheroidal and framboidal morphologies are only associated with microbial fossils and are present exclusively in the charophyte molds (rather than the zeolitic matrix) despite similar mineralogies in both the matrix and the vugs (identified via QEMSCAN), which supports the hypothesis that these morphologies are biomediated.

Distinct trace element suites have been recognized as a means of identifying biosignatures because organisms sequester trace elements during their lifetime and these suites can be preserved in minerals (Banfield *et al.*, 2001; Ferris, 2005). Correlation of elements from LA-ICP-MS in transects across putative biogenic features suggests the hypothesis that two suites of trace element associations (C, Fe, As and C, S, Se, P) can function as potential biomarkers (Table 2). Early analysis from the Mars Science Laboratory showed that C, S, and P are present in the rocks at Gale Crater, suggesting these rocks contain the elements necessary for life (Agle and Brown, 2013). The research presented here suggests that the presence of C, S, and P can function as a potential biomarker.

6. Conclusions

Diatoms and other algal fossils show degradation and some recrystallization of the iron (oxyhydr)oxides that precipitate on and around algal stalks; however, the morphological features are clearly recognizable on millennial timescales. Similar diatoms and algal fossils are present in the Brushy Basin Member, and these features meet all seven of the criteria supporting biogenicity in ancient rocks. Other possible biosignatures that occur in both the modern and ancient examples include biomediated spheroidal iron (oxyhydr)oxides and the presence of unstable 6-line ferrihydrite in 100 ka tufas and in Jurassic rocks. Modern and ancient examples exhibit potential chemical biomarkers of two suites of elements (1. C, Fe, As and, 2. C, S, Se, P) that may be unique to sequestration by microbes.

As the study of Gale Crater progresses, biosignatures in iron (oxyhydr)oxides such as framboids, spheroids, diagnostic trace element suites, and more amorphous mineral phases can serve as markers for possible preserved biosignatures where future explorations will endeavor to detect

past evidence of life. Mars Science Laboratory currently has the capacity to detect mineralogy and elemental composition, and future missions may include science payloads capable of higher-resolution trace element analysis and high-resolution microscopy. These terrestrial examples (1) show that iron (oxyhydr)oxide biosignatures as well as bona fide microbial fossils are preserved in Jurassic rocks, (2) show how microbial iron (oxyhydr)oxide precipitates persist and recrystallize to more stable forms during diagenesis, (3) provide a ground truth library of mineral textures, phases, and trace element configurations to explore and compare in extraterrestrial iron (oxyhydr)oxides (*e.g.*, in similar clay-rich shales in Gale Crater on Mars; Milliken *et al.*, 2010; Agle and Brown, 2013; Stack *et al.*, 2013), and (4) suggest the possible presence of a biomarker in the correlation of elements (C, S, and P) present in the rocks at Gale Crater.

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Abbreviations

LA-ICP-MS, laser ablation induced coupled mass spectroscopy; PCA, principal component analysis; QEMSCAN, Quantitative Elemental Mineralogy using SCANNing electron microscope; SEM, scanning electron microscope, scanning electron microscopy; XRD, X-ray diffraction.

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