Sedimentary Pyrite: A Window into the Microbial Past

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ABSTRACT

Microbes are ubiquitous in modern sediments, and must have been a similarly common constituent in the past. After death, however, they degrade readily and usually do not become part of the rock record. Especially for our understanding of early earth history and the evolution of life, however, finding preserved cellular remains has been critical. Verified microbial remains from early earth history (Awramik et al., 1983) have all been reported from sediments that experienced very early cementation by fine crystalline quartz (Schopf and Walter, 1983). The quartz cement renders these rocks (cherts) transparent and allows examination of microbial fossils in the context of the rock matrix (*3*). Because such cherts are rare, the microbial record of early as well as later earth history is still poorly known. Pyrite, another early diagenetic mineral that forms as a result of microbial processes in sediments, has so far received little attention as a source of microbial fossils. Pyrite grains ranging in age from Archean to Jurassic were examined by scanning and transmission electron microscope, and most of them show coccoid, rod-shaped, and even filamentous features that are interpreted as microbial. Although pyrite represents a much more common sedimentary mineralization than chert, its opaque nature has in the past rendered the search for contained microbial remains very difficult. The identification of microbial remains in sedimentary pyrite opens up the prospect to greatly expand our knowledge of microbial life in very old sediments, as well as allowing us much more detailed analysis of microbial life throughout Earth history. Sedimentary pyrite grains may also represent a good prospect to find traces of past life on Mars.

Introduction

For most of geologic history life on earth was all microbial, and as the eons passed linkages between the microbial biomass and geochemical cycles had a profound impact on the evolution of the atmosphere and oceans (Lovelock, 1988; Staley et al., 1997). Plants and animals may be foremost on our minds when we talk about the modern biosphere, but even today our planet's biomass is dominated by microbes (Staley et al., 1997). Due to their miniscule preservation potential, however, direct evidence of their past presence is hard to come by.

Microbial fossils are crucial for an understanding of early life history on earth, and fossilized microbes have been documented from Archean rocks as old as 3.5 Ga (Awramik et al., 19831). Notwithstanding several decades of searching, however, there is only a small number of localities with verified microbial remains of Archean age (Schopf and Walter, 1983). In all of these occurrences the microbial fossils occur in cherts (Schopf and Walter, 1983), silica cemented sediments that are preferred by students of early life because they ensure indigenousness of the microbial remains and allow their study within a transparent rock matrix (Schopf, 1999). In younger strata (Proterozoic and Phanerozoic) microbial fossils have been reported as well, and the best preserved examples are again found in occurrences of early diagenetic cherts. Because finding microbial fossils in cherts is still a matter of some luck, our knowledge of the earth's microbial record is spotty at best. Furthermore, because the cherts in question usually are associated with microbial mat deposits, the known microbial record is heavily biased towards microbes from shallow water photosynthetic microbial communities (Farmer, 1999).

For those of us that at one time or other have ventured to scoop up a handful of sediment from a tidal flat or a pond, the foul egg smell of hydrogen sulfide (H_2S) is probably

the most memorable experience that this material leaves us with. H_2S is produced by sulfate reducing bacteria that metabolize organic matter in the surface sediments, and its interaction with detrital iron minerals produces pyrite (FeS_2), a ubiquitous constituent of most marine sediments (Berner, 1984). Microbial sulfate reduction dates back to the early Archean era (Shen et al., 2001), and results in pore filling or concretionary pyrite grains that range in size from less than a mm to several cm's. This pyrite forms very early and close to the sediment surface, when the pore spaces of the sediment are teeming with microbes (Canfield and Raiswell, 1991; Bird et al., 2001). Because the preservation of soft tissue structures by pyrite is well known from the geologic record (Allison and Briggs, 1991), one might actually expect that the bacteria that live and thrive in the reducing sediments that favor pyrite formation may also be preserved in areas of pyrite precipitation. With pyrite being a ubiquitous early diagenetic mineral in all types of sedimentary rocks, there is an obvious potential here for recovery of microbial fossils from sedimentary rocks of all ages, and a range of environments.

Thus, while early diagenetic chert is certainly well suited to preserve microbial fossils, sedimentary pyrite clearly needs to be considered as an alternative source of microbial fossils. One of the drawbacks of pyrite is its opaque nature, which makes the search for enclosed microbial remains not as straightforward as in cherts. Schopf et al. (1965) were probably among the first to document preservation of microbes in sedimentary pyrite, using a transmission electron microscope (TEM). This discovery had little impact at the time, however, probably because the methodology used (carbon replicas) is very labor intensive, and also because few geologists are familiar with TEM’s. In addition, the use of carbon replicas also carries a certain risk for production of artifacts (Schopf, 1999).

Methods and Materials

To see how common microbial remains are in sedimentary pyrite, six small pyrite nodules from my specimen collection, ranging in age from Archean to Jurassic, were subjected to examination by SEM and TEM. Because sedimentary pyrite grains typically have an early, finer crystalline, nucleus that is overgrown by later diagenetic coarse pyrite (Canfield and Raiswell, 1991), the central portion of pyrite grains was targeted for study. Such a core portion was discernible in all nodules except the Jurassic one, which appeared to consist primarily of interlocking coarse pyrite crystals. For SEM study, specimens were first fractured and then a subset of fragments was etched with HNO^3 (etching time ranging from 10 to 120 seconds), rinsed with distilled water and allowed to dry. Unetched as well as etched specimens were coated with AuPd in a sputter coater and examined. For TEM examination, thin wafers of pyrite were prepared by polishing on diamond coated plastic sheets, attached to a sample grid, and ion-thinned for several hours to final thickness. After ion thinning the edge of the sample is only a few nanometers thick, and thickness increases gradually as we go away from the edge.

Results

In five of the studied pyrite grains, features interpreted as microbial remains have been observed. Only the Jurassic nodule did not yield bacterial remains. The observed features are described and illustrated in the following paragraphs.

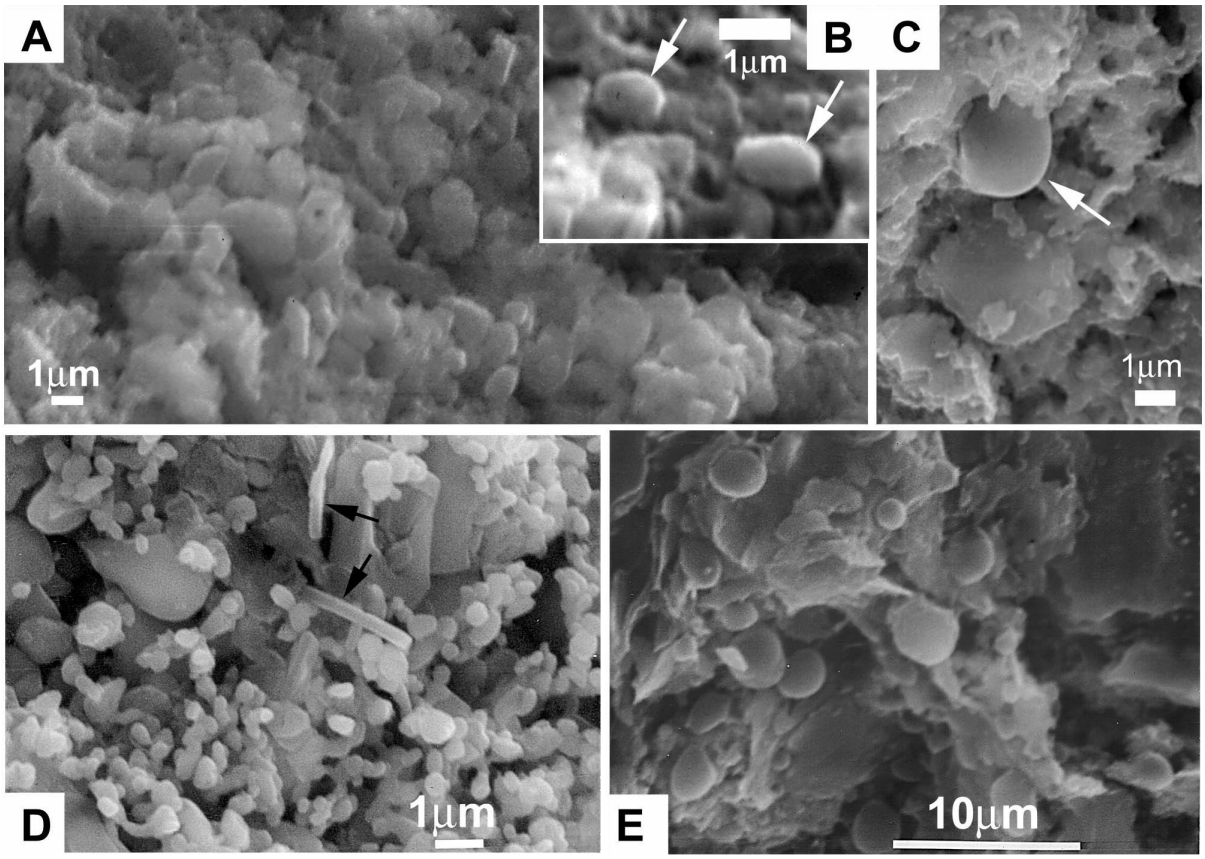

Figure 1: SEM observations. (A) Oviod pyrite bodies in etched pyrite from Ordovician Winnipeg Formation, Saskatchewan. (B+C) Higher magnification image of ovoid pyrite bodies (pointed out with arrows) seen in A. (D) Small spheroidal bodies and rods (black arrows) in pyrite grain from Middle Proterozoic Kaladgi Supergroup, India (not etched). (E) Spherical to elliptical pyrite bodies in etched pyrite grain from Late Devonian Chattanooga Shale, USA.

By shape and size the features in Fig. 1 and Fig. 2A strongly resemble coccoid bacteria, and even the smallest ones (Fig. 1D) are still in the size range of viable bacteria found in natural environments (Nealson, 1999). There is no need to assign the latter to the controversial nannobacteria category (Nealson, 1999; Folk, 1993). These rounded bodies contain iron and sulfur in the same proportion as the surrounding host pyrite (EDS analysis), and are therefore presumed to consist of pyrite as well. Due to etching, the host pyrite is heavily corroded. In Fig, 2A the lower ovoid is held in the corroded matrix at only a few points (white arrows), yet the ovoid itself has retained a smooth surface. The latter can also be seen in Fig. 1C, and E, and suggesting that the putative bacterial features are protected by an acid resistant membrane. The features in Figs. 2B and C resemble the remains of

filamentous microbes. Pyritization of microbial filaments has been described from other occurrences (Schopf, 1965; Rasmussen, 2000; Zierenberg and Schiffman, 1990), and trough like features seen in Fig. 2C, interpreted here as filament molds, have been described from microbial hot spring deposits (Jones et al., 2001) and modern stromatolites (Byrne et al., 2000).

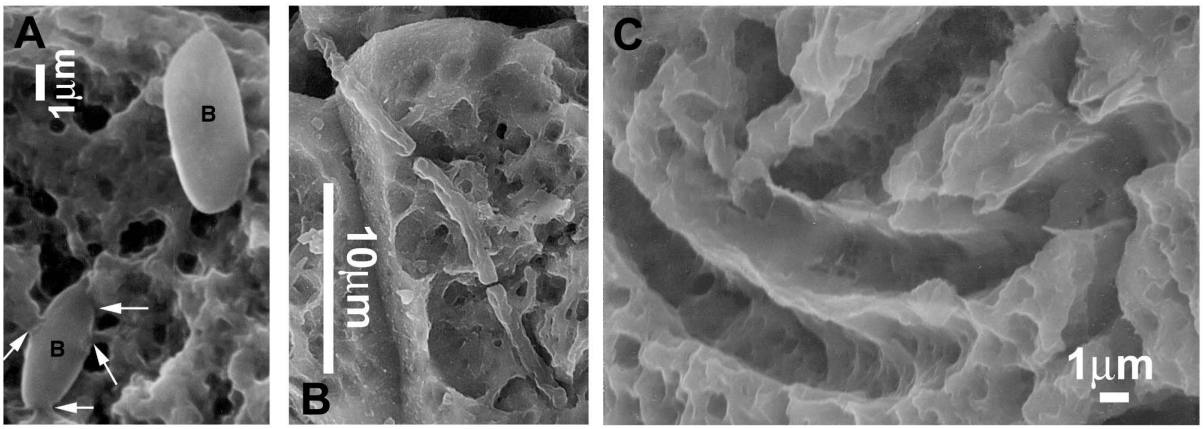

Figure 2: SEM observations. (A) Ovoid to rod-shaped pyrite bodies (marked B) in etched pyrite grain from Middle Proterozoic Belt Series, Montana, USA. (B) Pyritic filament on etched pyrite grain from Middle Proterozoic Kaladgi Supergroup, India. (C) Trough shaped molds from same (etched) pyrite grain as pictured in (A).

Figure 3 shows various rounded-oval shaped features observed under the TEM. In Figure 3C, carbon dominated chemical composition as well as a folded and wrinkled texture that resembles that seen in compressed sphaeromorphs, suggests a bacterial origin. Likewise, the observation that the pyritic core of the ellipsoidal feature in Fig. 3A differs by its lumpy texture from the surrounding pyrite matrix and is surrounded by a low-density carbonaceous membrane is suggestive of a microbial origin. The oval shaped pyritic body in Fig. 3B is probably a cross section of one of the rounded bacteriomorph pyrite bodies seen in Figs. 1A, B, and C.

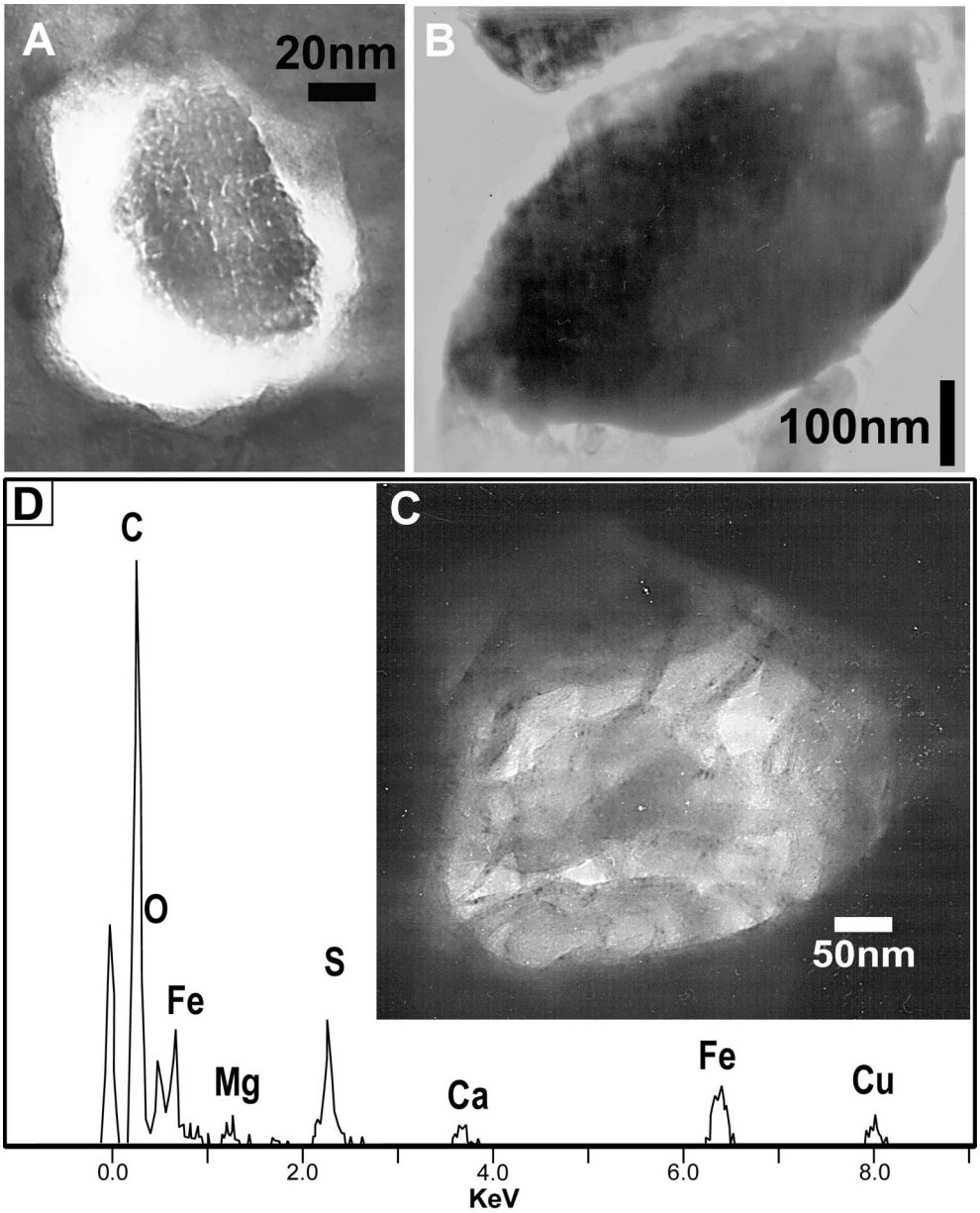

Figure 3: TEM observations. (A) ovoid feature in pyrite grain from Middle Proterozoic Belt Supergroup, Montana, USA. Dark center consists of pyrite (EDS) and its lumpy texture is absent in the surrounding matrix pyrite. This center is separated from the matrix by a rim of low density carbonaceous material (EDS). Features can reach several hundred nanometers in size and are very fragile (the larger one's tend to "drop out"). (B) Ovoid feature in pyrite grain from Ordovician Winnipeg Formation, Saskatachewan (same grain as in Fig. 1A, B, C). (C) Rounded low density feature in pyrite grain from the Late Archean Black Flag Beds of the Norseman region in southwestern Australia. Specimens up to 900nm were observed. Note the crumbled texture of the low density material, suggestive of a crushed or collapsed membrane. (D) EDS energy spectrum from the interior of the feature shown in (C). The material is amorphous and its high carbon content indicates an organic substance.

At first glance, the rounded cluster of pyrite grains in Fig. 4A (from a pyrite nodule in the Chattanooga Shale) appears to be a pyrite framboid, a feature that can be produced abiotically (Sweeney and Kaplan, 1973). Yet, whereas typical framboids, including those from the Chattanooga Shale (Fig. 4D, E), consist of euhedral crystallites (Butler and Rickard, 2001), the component grains in Fig. 4A have rounded outlines and show various degrees of distortion (Figs. 4B, and C). A texture like this could result when soft rounded sacs, such as bacterial cells, are impinging on each other. Also, in contrast to framboid crystallites that appear as single crystals under the SEM and TEM (Fig. 4D, E), the rounded pyrite grains in Fig. 4A are polycrystalline (Fig. 4C). These observations suggest that they did not form in the same manner as the "normal" pyrite framboids (Butler and Rickard, 2001). Figure 4F shows irregular-rounded pyritic bodies in a shale matrix that may also represent deformed bacterial cells, albeit with a more complex internal structure.

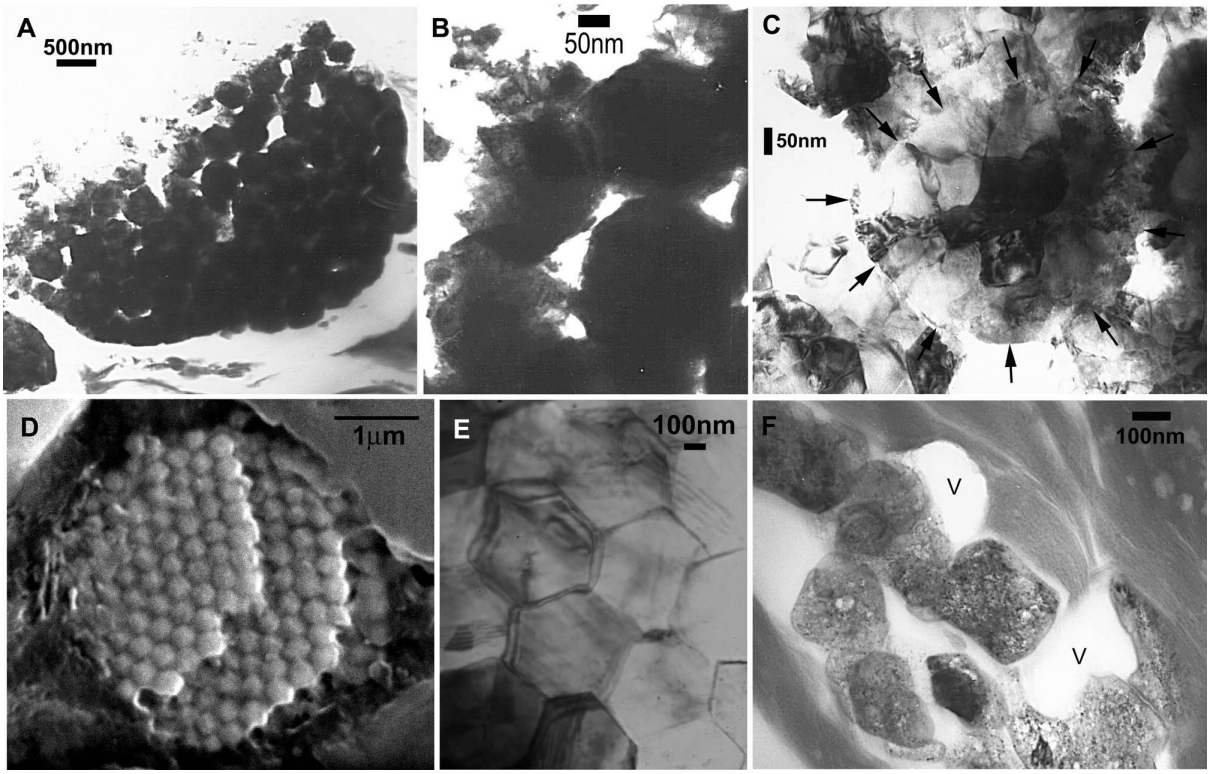

Figure 4: Bacteriomorph features in an early diagenetic pyrite concretion from the Late Devonian Chattanooga Shale, USA. (A) a cluster of rounded pyrite bodies (TEM). (B) closeup of pyrite bodies shown in (A), note rounded outlines (TEM). (C) higher magnification TEM image of pyrite bodies shown in (A). Arrows mark outline of rounded

pyrite grain. These pyrite grains are polycrystalline, as indicated by their mottled appearance (areas of different lattice orientation). This inference was confirmed with electron diffraction on single pyrite bodies. (D) a "normal" pyrite framboid from the shale that hosts this concretion. Note euhedral crystals. (E) The euhedral crystallites appear as single crystals under the TEM. (F) Cluster of irregular rounded pyritic bodies from shale inclusion in the same grain as pictured in (A). Spots marked V have been worn through by ion milling. These rounded bacteriomorphic bodies partially overlap and seem to have been pushed together by compaction (clay minerals aligned parallel to upper left – lower right diagonal).

Discussion

The features presented in Figs. 1, 2, and 3 exhibit a range of morphological traits common to microbes (filaments, cocci, rods) and are also in the expected size range. One may wonder, of course, how microbes could become entombed into a growing pyrite grain and maintain their morphological integrity. The study of modern sedimentary environments (Konhauser, 1998) as well as experimental work (Bubela and Cloud, 1983), however, shows that this indeed happens and may occur within a matter of weeks.

Provenance, age, indigenousness, syngenicity, and biogenicity are issues of concern when one looks for past life in the rock record (Schopf and Walter, 1983; Schopf, 1999). The first two requirements are satisfied because the specimens come from well studied successions with excellent age constraints. Samples were cut from drill core or very fresh outcrop specimens, no alteration rind of iron oxides or iron sulfate salts was detected on pyrite grains, and the observed features were fully enclosed within diagenetic pyrite. Thus the requirements of indigenousness and syngenicity are satisfied as well. Although it is understood that bacterial contaminants can make it into the freshest of subsurface samples, the reasons to argue against contamination in this study are as follows: (a) etching with nitric acid destroys bacterial contaminants; (b) bacterial remains are found within pyrite grains that show no signs of alteration/oxidation. Bacterial remains in Fig. 1D are from an unetched

sample, but they are unlikely to be contaminants because they are from within a freshly broken pyrite grain (no alteration detected), and also consist of pyrite (EDS analysis). Features observed with TEM (Fig. 3) were fully enclosed in a pyrite matrix prior to ion thinning, and must therefore be considered indigenous and syngenetic. Actually, the fact that pyrite is readily altered and destroyed in surface environments is an advantage in this context, because pyrite that was compromised by weathering processes is easily recognized.

The simple shapes of most microbes (Brock and Madigan, 1988) makes it easy to confuse them with rounded mineral grains, clumps of coaly matter, contaminating dust particles, and artifacts of sample preparation(Schopf, 1999). Because of the latter, SEM and TEM were in the past not considered reliable tools for the detection of microbial fossils (Schopf, 1999). Etching as well as application of conductive coatings can produce microbe-like artifacts in SEM (Kirkland et al., 1999; Bradley, 1999), although experimental work suggests them to be smaller than 100nm in size (Kirkland, 1999). The features in Figs. 1A, B, C, E, and 2A are unlikely candidates for etching artifacts because they are several microns in size and contrast strongly with the etched and corroded matrix pyrite. Furthermore, hydrothermal and late diagenetic cubic pyrite was etched in the same way the samples were, and no bacteriomorph artifacts were observed. Preparation of TEM specimens of pyrite by mechanical thinning, microtome, and carbon replicas can result in fracturing, distortions, and artifacts (Schopf, 1999). These problems, however, have been overcome with the development of ion mills. Gently wearing away the surface with a stream of argon ions, ion milling is ideal for this kind of study. Combination of SEM and TEM observation, ion milling of TEM samples, and focus on features in the bacterial size range (Nealson, 1999) strongly reduces the likelihood that artifacts and contaminants were mistaken for microbial remains.

Features that go beyond morphological resemblance to microbes, such as structural complexity and chemical characteristics are considered additional indicators of biogenicity (Schopf, 1999). For example in Fig. 3A, a lumpy-granular internal texture that differs from the surrounding pyrite matrix and an organic membrane increase the likelihood of biogenic origin. The observation that pyrite grains from this locality contain amino acid concentrations that are two to three orders of magnitude above that found in the surrounding host rock (Nardi et al., 1994), further favors a biogenic origin. The observation of organic membranes also suggests that the ovoids seen in SEM samples (Figs. 1A, B, C, E and 2A) remained smooth during etching because they were protected by such membranes, and thus supports the view that these ovoids are biogenic in nature. Filamentous forms (Figs. 2B, C), although they do not reveal individual cells, are very difficult to dismiss as simple etching artifacts.

Although several decades ago rounded clusters of pyrite grains were widely interpreted as the remains of bacteria (Love, 1957) and described as framboids, this notion fell out of favor when abiotic framboids were produced in the lab (Sweeney and Kaplan, 1973). Today, the abiotic nature of pyrite framboids is taken for granted. Yet, as pointed out above, the rounded pyrite grains seen in Figs. 4A, B, and C differ from those found in framboids in several important ways. Whereas framboid crystallites in nature and lab are euhedral (Butler and Rickard, 2001) and monocrystalline entities, the rounded pyrite cluster Fig. 4 consists of rounded-irregular and polycrystalline grains. This type of atypical framboids has been described in other studies of sedimentary microbiota (Javor and Mountjoy, 1976), suggesting that there may be abiotic as well as biogenic pyrite framboids in the sedimentary record. In the latter case, the clustering of rounded pyrite grains as seen in Fig. 4A would imply a higher level of structural complexity, interpretable as a bacterial colony.

One might initially think that the use of nitric acid as an etching medium would destroy above mentioned protective membranes. Yet, during burial diagenesis of sedimentary rocks the biopolymers present in bacterial cell walls undergo conversion to kerogen (Derenne et al., 1991), an organic substance that resembles polyethylene in composition and chemically inert nature (Petsch et al., 2000). That the kerogen in the carbonaceous host rocks of samples pictured here shares this property is indicated by the absence of visible oxidation when attempts were made to etch these rocks with nitric acid.

If one examines modern muds, they largely consist of loosely arranged sediment grains with abundant interstitial water (70-85%). Particles are coated with bacteria and bacterial slime, and in the pore spaces pyrite precipitation commences soon after deposition (Canfield and Raiswell, 1991). Within such muds, bacterial counts of 10^8 cells per gram or more are common (Ehrlich, 1990), and that some of the microbial inhabitants may become engulfed and entombed in early diagenetic pyrite should not come as a surprise and has been verified through experiments (Bubela and Cloud, 1983) and direct observations (Konhauser, 1998). Figures 1, 2, 3, and 4 vividly illustrate the wealth of bacteriomorphic features that can be found in sedimentary pyrite grains. What is even more remarkable is the fact that all of the early diagenetic pyrite grains examined in this study showed bacteriomorph features, regardless of geologic age (only the Jurassic nodule, consisting of late diagenetic pyrite cubes, did not yield microbial remains).

Conclusion

Although microbial preservation in pyrite was recognized many years ago (Schopf, 1965), the discovery had little impact at the time and the potential inherent in pyrite hosted

microbial fossils remained unrealized in the interim. As illustrated in this study, microbial remains in early diagenetic pyrite seem to be common in sediments from a wide range of ages and localities. Sedimentary pyrite therefore represents an as yet untapped source of information about the microbial geologic record. Because pyrite is such a ubiquitous sedimentary mineral there is a good chance that this record, once inspected carefully, will be essentially uninterrupted. In addition, whereas the chert-based microbial record is necessarily biased towards photosynthetic communities at the sediment surface, the pyrite hosted microbial record gives us a chance to finally examine the as yet largely unknown fossil record of anaerobic microbial communities. Furthermore, because pyrite is such a ubiquitous early diagenetic mineral in many marine successions, we have an opportunity to probe the geologic record of anaerobic bacteria in great detail. In the process we might for example recognize secular changes in anaerobic microbial communities that could influence the way we think about carbon and sulfur isotope shifts in the geologic record.

The recent prospect for traces of life on Mars (McKay et al., 1996) has sparked a lively debate not only on the methodology for identifying microbial remains (Schopf, 1999; Nealson, 1999; Bradley, 1999), but also on the strategies to get sample material with a high potential to contain such remains. Recent detail photos clearly show the stratified nature of the Martian surface (Malin and Edgett, 2000), as well as pronounced differential erosion. On Earth, the latter would suggest extensive soft mudstone strata, typically containing abundant early diagenetic pyrite grains. Considering the many similarities between Mars and Earth, one would expect that evolving life on Mars would likewise chance upon sulfate reduction as one possible metabolic pathway. Thus, to look for sedimentary pyrite grains in Martian mudstones might be a promising strategy to locate sample material with biogenic remains.

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Figures

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