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General palaeontology (Taphonomy and fossilisation)

Microfossils

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Abstract

Defining biosignatures, i.e. features that are indicative of past or present life, has been one of the major strategies developed over the last few years for the search of life on the early Earth and in the solar system. Current knowledge about microscopic remnants of fossil organisms, namely microfossils are reviewed, focusing on: (i) studies of recent environments used as analogues for the early Earth or extraterrestrial environments; (ii) examination of Precambrian rocks; and (iii) laboratory experiments simulating biotic and abiotic processes and resulting in the formation of genuine or pseudomicrofossils. Fossils' preservation depends on environment and chemical composition of the primary structure, although they might undergo taphonomic processes that alter their morphology and/or composition. Altogether, these examples illustrate what can be potentially preserved during the very first stages of fossilization and what can be left in the geological record after diagenesis and metamorphism. Finally, this provides a rationale to tentatively define diagnosis criteria for microfossils or ways to look for life on Earth or in extraterrestrial environments. ***To cite this article: E.J. Javaux, K. Benzerara, C. R. Palevol 8 (2009).***
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Résumé

Les microfossiles. La recherche de vie primitive sur Terre et dans les environnements extraterrestres requiert la caractérisation de biosignatures ou d'indices de vie. Cet article résume les avancées récentes de la communauté géobiologique sur les traces morphologiques microscopiques de vie : les microfossiles. En principe, les organismes appartenant aux trois grands domaines de la vie sont susceptibles d'être préservés sous forme de microfossiles. Cependant, suivant les conditions environnementales de préservation et les propriétés biologiques originelles, certaines formes de vie peuvent ne pas être fossilisées et d'autres voient leur morphologie et/ou composition chimique altérée(s), ou même détruite(s) par les processus taphonomiques. Les difficultés inhérentes à l'identification de microfossiles sont présentées, en s'appuyant, en outre, sur une série d'exemples de recherches géobiologiques menées sur des environnements actuels analogues aux conditions de la terre primitive ou d'autres corps du système solaire, sur des roches précambriennes et enfin dans le cadre d'expériences en laboratoire explorant les processus biotiques et abiotiques. Les

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éléments de diagnose nécessaires pour identifier des microfossiles dans des roches, utiles pour la micropaléontologie terrestre et l'exopaléontologie sont discutés. ***Pour citer cet article : E.J. Javaux, K. Benzerara, C. R. Palevol 8 (2009).***
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Keywords: Microfossils; Geobiology; Taphonomy; Biogenicity; Endogenicity; Syngeneity

Mots clés : Microfossiles ; Géobiologie ; Taphonomie ; Biogénicité ; Endogénicité ; Syngéneité

1. Introduction

The search for life on early Earth and beyond Earth in the solar system requires the characterisation of biosignatures (traces or indices of past or present life). Biosignatures have traditionally included chemical, isotopic and morphological proxies that have been interpreted as remnants of life-preserved in rocks [10]. Morphological signatures can be macroscopic such as macrofossils, or sedimentary structures built by microorganisms, such as stromatolites. However, this article focuses more specifically on the microscopic morphological signatures of life which are generally called microfossils. Microorganisms can produce biominerals that might have a particular chemistry, crystallography, and/or texture, but these are more specifically discussed by Benzerara and Menguy (*Looking for traces of life in minerals* [this issue]). They can also alter rocks or minerals and leave microchannels such as those formed by endolithic cyanobacteria in carbonates, e.g. [29], or in basalts [25] but these "ichnofossils" or traces of biological activity are not microfossils themselves.

On Earth, the only reference planet inhabited by life that we know, one common feature of life, is the cell (with the exception of viruses). The three domains of life include cells with diverse biochemical properties that are important for their preservation potential in the fossil record. When looking for traces of early life in terrestrial rocks, we have to consider three important issues [10]:

- *the preservation environment*: under what conditions are cells with varying biochemical properties preserved?
- *the taphonomy*: how do processes of degradation and preservation retain, alter or erase original biological properties?
- *the criteria of biogenicity*: how can we tell biological from non-biological when observing purported microfossils in rocks?

Here we shortly review a series of examples from geobiological investigations that were carried out: (1) modern environments considered as analogues of some environments of the early Earth or other planets; (2) Precambrian rocks; and (3) laboratory systems that simulate biotic and abiotic processes forming microfossils. These examples will help in defining microfossils, as well as understanding the mechanisms of fossilisation, including the processes that potentially erase biosignatures. From there, it will be possible to discuss criteria of biogenicity that may be applicable to Earth systems but also will be useful for exopaleontology.

2. What is a microfossil?

2.1. Definition

Microfossils are the microscopic remains of organisms. The organisms may be prokaryotic cells of the Bacteria or Archaea domains, unicellular eukaryotes (protists), whole multicellular eukaryotes, or parts of multicellular microscopic or macroscopic eukaryotes. Virus can be included, although so far only very few reports suggesting their presence in the fossil record exist e.g. [46]. The size of a microfossil ranges from the smallest living cell (250 ± 50 nm constitutes a reasonable lower size limit for life as we know it, [39]) to larger sizes that are not visible with the naked eye.

2.2. Composition

Microfossils can have a variety of morphology and chemical composition, depending on their original properties and the conditions in which they are preserved (Fig. 1).

2.2.1. Carbonaceous composition

The organic sheaths of cyanobacteria and the walls of microscopic unicellular and multicellular algae, fungi, diverse protists like dinoflagellates, thecamoebians, and plant spores or animal eggs can be preserved as carbonaceous objects in fine-grained sediments. Because of the compaction of enclosing sediments, these micro remains are usually flattened and their walls show wrinkling, folding, or breaks resulting from mechanical stresses. Such taphonomic features modify the original size and morphology of the organisms, but they can

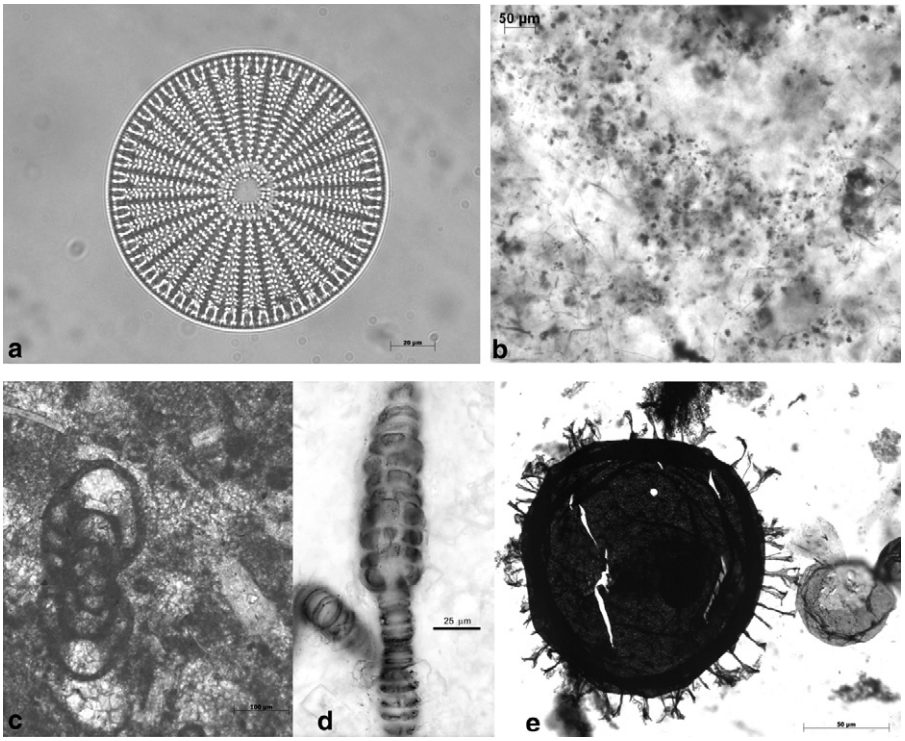

Fig. 1. Examples of organic-walled and mineralised microfossils in diverse preservational modes. a: siliceous diatom frustule; b: assemblage of coccoids and filaments coated by iron oxides and permineralized by silica in ∼1.9 Ga Gunflint stromatolite, Canada; c: calcareous benthic foraminifera preserved in Carboniferous carbonates, Belgium; d: ∼1.2 Ga multicellular bangiophyte algae preserved tri-dimensionally in chert, Hunting Fm, Canada (picture courtesy of N. Butterfield); e: ornamented organic-walled microfossil (acritarch) preserved flattened in 2D in shale, ∼1.3 Ga Ruyang Group, China.

Fig. 1. Exemples de microfossiles à paroi organique et à paroi minérale dans divers modes de préservation. a : frustule siliceux de diatomée ; b : assemblage de coccoïdes et filaments couverts d'oxydes de fer et perminéralisés par la silice dans les stromatolites de la Formation Gunflint, Canada (∼1.9 Ga) ; c : foraminifère benthique calcaire préservé dans des calcaires du Carbonifère, Belgique ; d : algue rouge Bangiophycée préservée en trois dimensions dans des cherts de la Formation Hunting, Canada (∼1.2 Ga) (photo de N. Butterfield) ; e : microfossile à paroi organique ornementée (acritarche) comprimé en deux dimensions dans des shales, du Groupe Ruyang, Chine (∼1.3 Ga).

help the paleobiologist to differentiate biogenic from abiogenic carbonaceous material. Indeed, fine abiogenic or biogenic kerogen particles, or bitumen droplets, can agglomerate and produce carbonaceous spheres whose biogenicity is difficult to assess when preserved in 3D. The flattening in 2D of a carbonaceous vesicle with associated taphonomic features, and their resistance to acid maceration (for extraction from the rock matrix) show the integrity of genuine organic-walled vesicles and permit to discriminate them from agglomerated organic particles (Fig. 1).

The organic remains might be preserved in three dimensions if, after the death of the organism, they are rapidly entombed in ice, amber (for relatively young organisms on the geological timescale, i.e. up to 40 kyrs) or in silica (chert), and are thus protected from subsequent degradation by microorganisms. Similarly, sorption (i.e. adsorption or precipitation) of metals onto sheaths, cell walls, or microbial exopolymers may play the same preservation role for trapped organic matter (see [43] for detailed explanations). Finally, some carbonaceous polymers are very resistant to chemical degradation (e.g. sporopollenin forming the cell wall of plant spores for example) and are thus often preserved despite diagenesis.

The chemical composition of the molecules composing the cell walls or sheaths of microorganisms is usually transformed significantly during metamorphism. For example, volatiles such as H, N are lost while the aromatic carbon content increases with temperature. Theses changes can be observed by optical microscopy, as they correspond to a change of colour of the organic-walled microfossil from yellow, to orange then brown and finally black. This colour change corresponds to different stages of organic matter thermal maturity and colour charts have been developed classically by palynologists for the oil industry e.g. [2]. This changing thermal maturity can be associated over a certain temper-

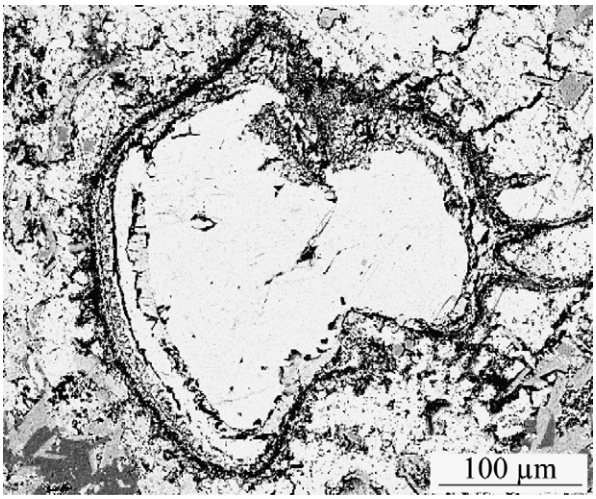

Fig. 2. Scanning Electron Microscopy (Backscattered electron mode) image of a lycophyte spore in high-grade metamorphic rocks from the Vanoise massif (Alps, France). These rocks experienced a peak pressure of ∼14 kbars and a peak temperature of ∼360 °C, corresponding to a burial of ∼35 km. The preserved carbonaceous cell wall of the spore appears in black. Some chemical signatures of the organic polymer composing originally the cell wall of the spores, i.e. sporopollenin, can still be detected in the metamorphosed rocks [7]. This highly resistant polymer forms specific ornaments in the cell wall.
Fig. 2. Image au microscope électronique à balayage (mode en électrons rétro-diffusés-BSE) d'une spore de lycophyte dans des roches à grade métamorphique élevé du massif de la Vanoise (Alpes, France). Ces roches ont subi une pression maximale de ∼14 kbars et une température maximale de ∼360 °C, correspondant à une profondeur d'enfouissement de ∼35 km. La paroi cellulaire préservée de la spore apparaît en noir. Des signatures chimiques du polymère organique composant la paroi des spores, la sporopollénine, peuvent toujours être détectées dans les roches métamorphiques [7]. Ce polymère très résistant forme des ornementations spécifiques sur la paroi.

ature with an increasing degree of graphitisation of the OM that can be estimated by Raman microspectroscopy [7] (Fig. 2).

2.2.2. *Mineral composition*

Microorganisms can produce minerals that eventually facilitate their preservation, such as calcium carbonates precipitated onto cyanobacterial sheaths (i.e. fibrous polysaccharide envelopes forming outside the cell wall), calcium phosphate of conodonts, silica frustules of diatoms... (Fig. 1). In this case, the mineral composition of the microfossil wall is primary. Biominerals are discussed in another paper in this issue by Benzerara and Menguy. Alternatively, the mineral composition can be secondary, the original carbonaceous or mineral walls or envelopes being in that case partially or completely replaced by other minerals. These minerals such as pyrite, iron oxides, silica, calcite, and phosphate may form casts or molds of micro remains during diagenesis, following dissolution or permineralisation of the original structure.

For example, microbial cells can be fossilized by silica in silica-rich fluids such as in hot springs or vents resulting in the preservation of very fine structures of the cells as well as some of the organic content of the cells e.g. [66]. Similarly, calcium phosphates can replace exquisitely the original organic wall of Neoproterozoic testate amoebae [56] or of early animal eggs [72]. Recently, it has been shown that iron-oxides can precipitate on and into virus capsids or microbial cells and sheaths in vents, acidic rivers or Fe-rich anoxic environments [20,22,53].

3. Processes of fossilisation: examples

The three domains of life can, in principle, be preserved as microfossils, depending on the conditions of preservation, and their original composition. A wide diversity of processes can be involved in the fossilization of cellular structures in association with the diversity of preservation environments. To illustrate this diversity, we present here a non-exhaustive list of examples collected from studies on recent and ancient environments, and in the laboratory.

3.1. *Some examples of recent preservation environments*

The Rio Tinto river (Spain) is an acid mine drainage characterized by a very low pH (frequently near or below 1) and the intensive precipitation of iron oxides and sulphates that can entomb cell remains [19,20]. This environment has been proposed as a geochemical and mineralogical analogue to Terra Meridiani on Mars, even though the physical processes of rock formation differ significantly between the two sites. Another interesting feature of the Rio Tinto is the presence of 1 or 2 Ma old terraces permitting a direct comparison between past and recent geobiological processes acting in the river. Coccoidal and filamentous bacteria, algae and fungi are preserved locally as casts and molds in laminated ironstones, even when they experienced diagenetic transformations, such as the transformation of goethite into hematite [19,20]. More generally, bacteria can trigger mineral formation in any environment that is saturated with respect to a specific mineral phase, at least locally and even temporarily around the bacterial cells. Mineral precipitation by microorganisms can be achieved in a wide range of physico-chemical conditions, and by various passive or active processes that are reviewed in details by Konhauser [43]. In oxic sediments

forming in neutral-pH freshwater lakes, natural bacterial exopolymers and cell walls can become covered with very thin nanoscale elongated crystals of poorly ordered iron oxides [22,53] leading eventually to the preservation of iron-organic carbon assemblages with approximately the same morphology as the original microbes.

Environments such as glacial lakes, melt waters or rocks in the Dry valleys of Antarctica are also inhabited by diverse microbes including cyanobacteria, algae, or fungi. Cyanobacteria and fungi live in pores of the McMurdo Dry valleys sandstones. After death, their cells decay and their cell walls and sheaths are covered by allochtonous clay minerals and sulfate-rich salts filling the sandstone pores while the empty moulds of cells are filled by minerals. The organic cellular structures serve as templates for diffusing mineral elements and give rise to a characteristic distribution pattern of precipitates inside the fossilized cells. In the absence of organic matter preservation, the former presence of these cells and their mineralization impart a special texture to the rock with formation of cell-shaped structures [70].

In Iceland hot springs, intra- and extra-cellular silicification is due to the polymerization of silica from hydrothermal fluids. Only microbial sheaths are usually preserved. Although silicification can alter the morphology of the microbes beyond identification, it has been proposed that microorganisms impart an influence on the fabric of the siliceous sinters that form around hot spring vents. Indeed, this fabric consists in alternating laminae of flat-lying and upright filamentous microorganisms resulting from the behaviour and motility of living cells [44,45].

3.2. Examples from past environments

In Greenland, Neoproterozoic carbonate tidal flats preserve mats bearing the cyanobacteria *Entophysallis*, which have a mammillate (pustulate) surface similar to modern mat surfaces built by the same organisms in Abu Dhabi sabkha. At the microscopic level, microfossils are preserved by the permineralization of their cell walls by silica, which leaves some remains of organic matter along the cell walls. Some colonies even show patterns of pigmentation in the most external layer of cells. These are interpreted (by comparison with modern analogues) as the fossil remains of pigmented cells most exposed to the light and UV [30,31,41]. Remarkably well-preserved coccoids and sheaths of microbes are abundantly present in stromatolitic and non-stromatolitic cherts (i.e. silica rich sedimentary rocks) of the 1.9 Ga Gunflint iron formation (Canada). It has been shown that these cherts precipitated directly on the seafloor, and did not result from secondary precipitation of silica nodules within carbonates. The Gunflint stromatolites resemble hot spring sinters like those existing presently in Yellowstone, and differ from past and modern tidal flats stromatolites in which the microfossils are usually mat builders. This can be in particular inferred from the fact that the fossils in Gunflint stromatolites are jumbled together without preferential orientation (no dense intertwined bundles of filaments). The fossils are coated by iron oxides and permineralized by silica, with partial preservation of organic walls [40] (Fig. 1).

Proterozoic shales from peritidal to basinal marine environments show exceptional preservation of organic-walled microfossils e.g. [14,32–34,42]. The fossils are preserved in 2D (flattened) rather than in 3D as in chert, but exquisite details of the ornamentation and the ultrastructure of the fossil walls can be observed which may provide key information on their taxonomy [34,65].

3.3. Actualistic studies

Conducting experiments in the laboratory offers a complementary approach to investigate taphonomic processes (i.e. processes occurring after the death of the organisms) and decipher what can be preserved in microfossils, regarding their chemistry as well as their ultrastructure. Although these studies may not all reflect the full complexity of natural environmental conditions, they reveal biotic patterns and preservable properties that have been overlooked so far. Fossilization, i.e. the stabilization or replacement of organic structures by mineral deposits, is indeed a very quick process that can be achieved in few hours or days. For example, exposing bacterial cells to a solution rich in Ca, Fe or Si can lead to their encrustation in few hours [4]. If precipitation occurs in close connection with the microbial structures and if the minerals are small enough, very fine cellular details can be preserved. For example, the 40 nm thick cell wall of Gram-negative bacteria can be fossilized by calcium phosphates e.g. [4] or iron minerals e.g. [53] (Fig. 3). Embryos of eukaryotes could be preserved as well in the laboratory at different development stages by phosphatization, simulating what likely occurred during the fossilization of the famous ~630 Ma old Doushantuo embryos e.g. [73]. While some organic carbon might be degraded during these processes, it has been shown that most of it can be preserved within the resulting mineralised microfossils e.g. [4]. After the precipitation of minerals on the organic structures, further degradation of the morphology and degradation/maturation of the organic remains can occur. This stage is influenced by mechanical stresses, circulation of fluids, and metamor-

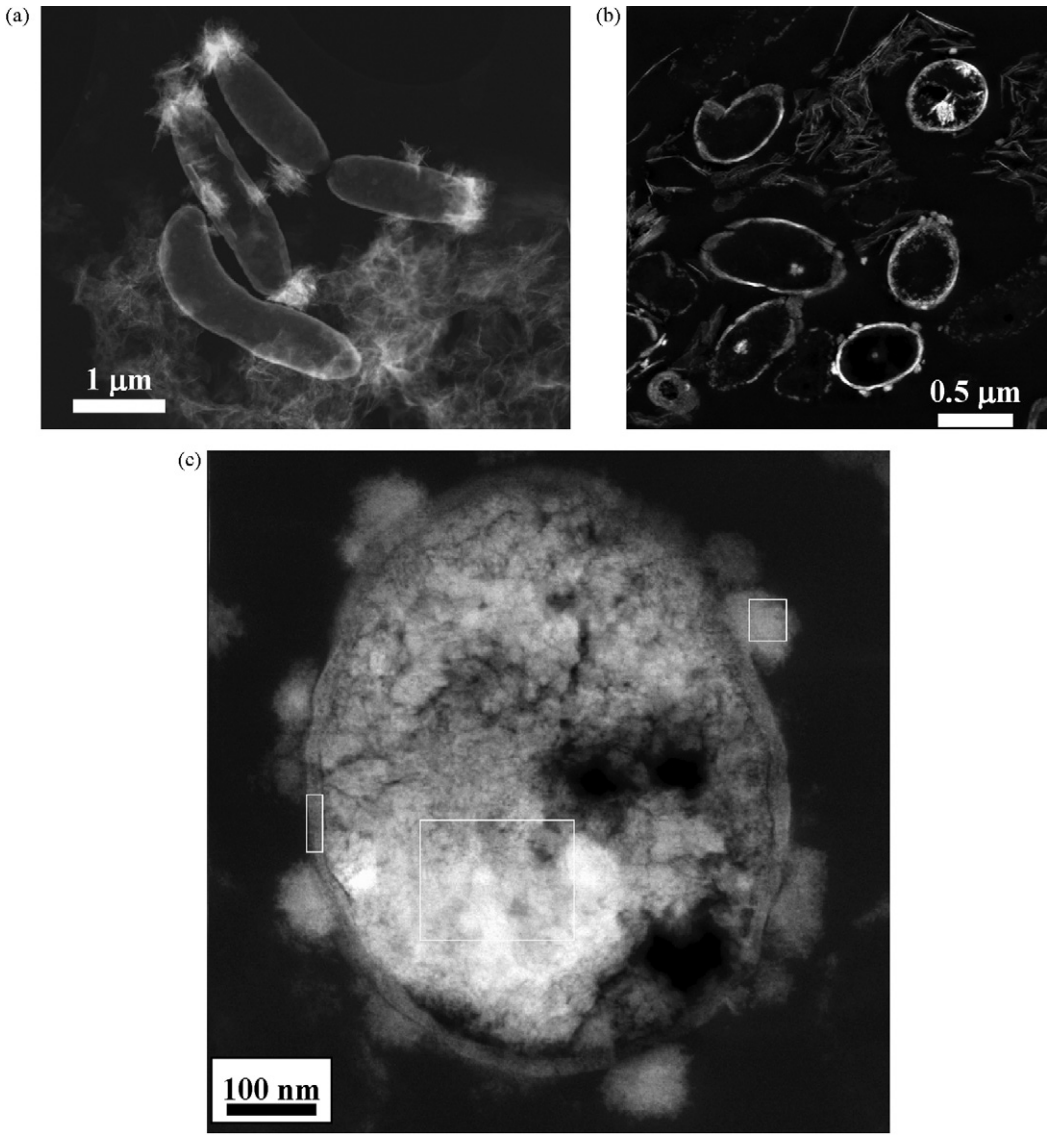

Fig. 3. Transmission Electron Microscopy (High Angular Annular Dark Field mode) images of experimentally fossilized Fe-oxidizing bacteria [53]. Bright areas are rich in Fe. (**a**): image of whole bacterial cells cultured for one day in a Fe-rich medium. Cells are systematically outlined by a thin Fe-rich layer. Extracellular precipitates appear as fluffy clusters of Fe-rich phases. (**b**) and (**c**): images of ultramicrotomy ultrathin sections (∼70 nm in thickness). The iron-rich precipitates forming within the cell walls of the bacteria are clearly visible. They form layers that are 40 nm in thickness. Cells are sometimes filled secondarily by precipitates as observed in (**c**). Extracellular Fe-rich globules sometimes also grow at the surface of some cells.

Fig. 3. Images au microscope électronique à transmission (mode *High Angular Annular Dark Field*) de bactéries ferro-oxydantes fossilisées expérimentalement [53]. Les zones brillantes sont riches en fer. (**a**) : image des bactéries entières cultivées pendant un jour dans un milieu riche en fer. Les cellules sont systématiquement entourées d'une fine couche de fer. Les précipités extracellulaires apparaissent comme des amas touffus de phases riches en fer. (**b**) et (**c**) : images de sections ultrafines (∼70 nm d'épaisseur). Les précipités riches en fer se formant dans les parois des bactéries sont clairement visibles. Ils forment des couches épaisses de 40 nm. Les cellules sont parfois remplies secondairement de précipités (**c**). Des globules extracellulaires riches en fer poussent parfois aussi à la surface des cellules.

phism. Observations of natural samples have shown that microfossils can sometimes be preserved even after high grade metamorphism e.g. [7] (Fig. 2). Such processes take place over much longer timescales, so it is more difficult to simulate them in the laboratory. However, some recent studies provide an interesting way to address this issue, in particular by noting that time and temperature are inherently linked and that aging at low temperature over long timescales can be simulated by shorter aging at a higher temperature (see [63]).

Many experimental taphonomy simulations can be found in the literature. Cyanobacteria have received a particular attention. Intra- and extra-cellular silicification was observed. It has been shown that sheaths are preferentially preserved to walls and cytoplasms e.g. [1]. Some morphological changes could be observed including cell collapse, shrinkage and disappearance, trichome disarticulation, varying terminal cell morphology, and shrivelling, constriction/swelling, discoloration, rupture of sheath e.g. [66]. Although virus may have been major players in life evolution e.g. [21], only little is known about their geological record. Interestingly, some taphonomy experiments have been performed on these "organisms". Biomineralisation experiments on viruses show that dissolved iron ions are able to penetrate the virus capsids and bind to internal sites [17]. As a result, virus capsids can serve as nuclei for the growth of iron oxide particles. The resulting morphology differs from abiotic iron oxides and organic molecules composing originally the capsids can be efficiently preserved by the iron minerals that armour them offering the possibility to look for them e.g. [37].

3.4. Abiotic processes and products

One key problem encountered in the study of microfossils is that relatively complex morphologies can be produced by purely abiotic processes (e.g. [28], Livage, this issue) and can thus make their identification difficult. Laboratory experiments can be of interest in order to look for possible specific features (if any) that might be used to discriminate abiotic from biological objects. In addition to the morphology, the composition of the organic matter produced by reactions such Fischer-Tropsch has been carefully scrutinized and compared with mature kerogens e.g. [52]. For example, a combination of infra-red spectroscopy (providing an aliphaticity index of the organic matter) and microscale measurements of the carbon isotopic compositions was used by Sangely et al. [58] to distinguish between biology and Fischer-Tropsch-type reactions as genetic processes for the bitumen found in the Cretaceous uranium deposits of Athabasca. Known abiotic products that can mimic life morphologies or chemistries include vesicles made in the laboratory from meteoritic kerogen or in other prebiotic chemistry experiments e.g. [18], fluid inclusions, carbonaceous filamentous shapes resulting from migrating organic matter (with carbon isotopic fractionation resembling life patterns) around minerals casts in hydrothermal environments [11,12], aggregates of silica spheres and rods in silica-rich waters of hydrothermal springs, migration of carbonaceous materials along microfractures [68] or around silica spheres formed in silica-saturated water (these are less than 5 μm in diameter and formed in hydrothermal conditions, e.g. [36]). Mineralised pseudo-fossils have been produced using a mixture of barium carbonate and silica in laboratory experiments [28]. The resulting auto-assembling segmented filaments were rigid tri-dimensional objects, not hollow originally, but made hollowed and filled by organic matter during further experiments. In principle, these artificial objects could form in nature and be confused with fossils or biominerals. However detailed observations of the septae morphology, the population morphology and distribution in the rock may alert the micropaleontologist. For example, such objects would never be found in fine-grained siliciclastic sediments where filaments are preserved as carbonaceous objects flattened in two dimensions.

Further studies are much needed to investigate the range of abiotic processes mimicking life properties, to define unambiguous traces of life for paleobiology but also for the search of life beyond Earth.

4. How to tell biological from non-biological?

Several criteria have been proposed in the literature in order to test the biogenicity of microstructures preserved three-dimensionally in cherts or silicified carbonates [10,11,13,30,31,59–62,64,69]. Most of them underline the importance of proving the endogenicity, the syngeneity, and the biogenicity of the microstructures in question in a well-characterized geological context. Others insist on an additional falsification approach where all possible abiotic hypotheses should be excluded before a biological origin can be accepted [11,12]. We briefly explain those criteria below.

4.1. Geological context

The first step in the recognition of past traces of life is the determination of the paleoenvironmental conditions of preservation. The samples should come from rocks of known provenance, of established age and demonstrating geographic extent. Moreover, the possible traces should occur in a geological context plausible for life: these criteria apply mostly for sedimentary environments. It has been traditionally inferred that magmatic and metamorphic rocks were not appropriate for the search of fossils as the former form under conditions incompatible with life and the latter have experienced temperature and pressure conditions that might have erased any trace of life. However, recent studies have suggested that even

basalts can be altered by life e.g. [67] and might retain traces of this bio-activity although there is still some discussion e.g. [6,23–25]. Moreover, it has been shown that fossils of plant spores could be very well preserved in rocks that have experienced high grade metamorphism, ~14 kbar and 360 °C, i.e. a burial of ~35 km [7].

4.2. Criteria for endogenicity

Rocks even present at depth can be colonized secondarily by recent microorganisms through fractures for example. It is thus important to show that microfossils are clearly endogenous to the rock, i.e. enclosed within the minerals composing the rock or between the grains before cementation of the rock. They should be observed in thin sections cut in the rock perpendicular to bedding. If the microfossils are organic-walled and preserved in two dimensions in fine-grained siliciclastics, following sedimentation with other fine particles, additional thin sections parallel to bedding will show them more clearly (in cross-cutting sections, they appear as a fine brown/black streak). The repeated use of *in situ* analytical techniques to study microfossils within rock samples allow ascertaining that those objects do not result from contamination/artefacts produced during sample collection or preparation. Mineralised microstructures should also be shown in situ in thin sections, and their primary occurrence should be demonstrated by petrologic analyses proving early cementation as other grains making up the rock or active building of the rock structure (as in some stromatolites; e.g. [57]). Finally, the possibility to observe the interface between microfossils and their hosting minerals down to the few nanometer-scale can help unravelling possible chemical interactions between them and prove further the endogenicity of the objects [8]. Endogenicity is however not sufficient to ascertain that the object that is observed is a microfossil. Indeed, some microorganisms, called endolithic microorganisms can bore into rocks and live inside. They have been in particular abundantly documented in carbonate deposits and stromatolites e.g. [16]. Hence, one can potentially find a microbe younger than its hosting rock. Their actual significance in the geological record still remains to be evaluated.

4.3. Criteria for syngeneity

Most of the time, microfossils cannot be dated directly and their age is thus inferred from the age of the host rock. It is thus important to prove that microfossils have the same age as the rock itself, i.e. they occurred in the sediments prior to their diagenesis and lithification (syngeneity). Some criteria can be proposed: there should be no hydrothermal veins or fractures cutting through the studied samples as they are preferential path for the input of exogenous abiotic or biotic organic compounds in the rock. Endolithic microorganisms are another issue as explained above. One way to test this is to perform Raman spectroscopy analyses on isolated microfossils to assess their thermal maturity [9] and check that it is consistent with the thermal maturity of other carbonaceous particles found within the rock and the metamorphic grade recorded in the minerals composing the hosting rock [48,50]. The hypothesis of contamination by younger material may then be discarded.

4.4. Criteria for biogenicity

As mentioned above, abiotic processes can form relatively complex morphologies similar to those produced by life. In addition to morphology, some criteria have thus been proposed in the literature e.g. [15]. The microstructures should be relatively abundant (a population), have a biological morphology (comparable to that exhibited by modern microorganisms or well-documented fossils from younger successions) and have biological size ranges (larger than the smallest extant free-living organisms (>0.01 μm^3). The past debate on the existence of purported nanobacteria was of particular interest regarding the size criterion. It has been clearly shown that those objects, too small to be bacteria, were indeed not microorganisms despite their biological-like shapes e.g. [3,54]. Mineralized microstructures should exhibit morphologies that are relatively complex, even though we note that this notion of complexity has not been yet clearly defined in the literature. For example, it is difficult to distinguish silica spheres representing mould of bacterial cells or abiotic chemical precipitates, unless they are hollow and contain traces of endogenous kerogen. Moreover, some abiotic processes can mimic so-called complex biological morphologies e.g. [27]. Thus, microstructures with simple morphologies, i.e. without ornamentation (decoration of the wall such as processes, striation, polygonal networks, pustules, vesicles, chagrinate pattern...) should ideally show a structurally distinctive carbonaceous cell wall. Organic-walled microstructures may show cell lumina (originally cytoplasm-filled cell cavities) although this is difficult to observe in flattened specimens in thin sections through compacted mud or silt but more easily seen in 3-D preserved fossils. They should show evidence of taphonomic degradation, such as thin concentric folds or lanceolate folds, or folding over, and, if preserved in fine-grained sediments, flattening in two-dimensions,

demonstrating the flexibility of the original wall. Several techniques provide key information on the composition of possibly biogenic, carbonaceous particles: for example, Raman microspectroscopy e.g. [35,38,61,71,74], Infra-Red microspectroscopy e.g. [49], Scanning Transmission X-Ray microscopy e.g. [5,48] or carbon isotope spectrometry e.g. [26]. However, none of these techniques gives a definitive answer to the biogenicity of carbonaceous matter. For example, it has been clearly mentioned by Pasteris and Wopencka [55] that Raman spectroscopy of carbonaceous material does not provide definitive evidence of biogenicity by itself. Analyses of the carbon isotope fractionation of the bulk kerogen should reveal negative values that are consistent with a biogenic interpretation. However, such values can also be produced by abiotic processes [51]. The biogenicity of microstructures is further strengthened by the observation of their distribution in the rock and fossilized behaviour: orientation and distribution caused by mobility and interaction with the environment, the presence of pigmentation at colony surfaces, and cellular division e.g. [13,31,41,47].

4.5. Falsification

Noting the difficulty of discriminating unambiguously biogenic from abiotic objects, Brasier et al. [12] propose a different approach consisting in demonstrating that an object cannot be abiotic instead of trying to find biogenic signatures: this is the falsification approach. As underlined above, abiotic processes can produce microstructures mimicking biological morphologies and sometimes chemistries (see section 3.4). They include migration of abiotic (or biotic) carbonaceous material along microfractures, around mineral casts, or around silica spheres formed in silica-saturated water. The formation of filaments in which abiotic carbonaceous matter is intimately associated with minerals has been shown in the laboratory [27]. These structures may be recorded in the rock record, and they may be preserved in three dimensions in carbonaceous chert (as the SOS-self-organizing structures – described by Brasier et al. [12]). In this regard, the taphonomic processes associated with preservation in fine-grained siliciclastics such as flattening in two dimensions, folding and other soft wall deformation of carbonaceous microstructures help the paleobiologist to determine their biogenicity.

5. Conclusions

The unambiguous determination of microstructures as microfossils is a difficult task, especially in rocks of Archean age, in which the earliest traces of life can be found. As discussed above, a set of criteria involving a multidisciplinary approach is necessary to establish the biogenicity, endogenicity and syngeneity of a microstructure with high confidence. The taphonomic processes differ depending on the preservational environments and the original biology, leading to biases in fossil diversity and morphology, often preventing identification or even recognition. However some criteria presented here can be pinpointed at the microscopic levels, within a well-understood geological context.

For simple life forms, morphology alone is not sufficient for determining biogenicity, but needs to be combined with studies of populations (large fossil assemblage), the particle distribution, biogeochemistry, degradation patterns and the knowledge of the geological environment, whereas when morphology (and biogeochemistry) is more complex, a taxonomical approach can be performed. We note that deciphering the biogenicity of an object is very difficult even using cutting-edge *in situ* techniques. Moreover, comparing with our only known example of life, the appearance of morphologically complex organisms takes time and requires special conditions (biologically-produced oxygen) so that such fossils are therefore improbable to find in the Solar System. This emphasizes the difficulties of finding unambiguous traces of life on other planets without being able to take samples back to Earth.

Geobiological studies in recent and past environments and laboratory experiments can improve our understanding of preservational environments and taphonomic processes, and help us to recognize traces of life on early Earth and beyond Earth. We can then predict possible past or present extraterrestrial habitats and make predictions of the types of traces of life (“biosignatures” or better, “indices of life”, see the introduction chapter) that could be preserved in these environments. This is essential to choose landing sites, instrumentation, and samples to return in exobiological missions.

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