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Bacteria in the Tatahouine meteorite: nanometric-scale life in rocks

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Abstract

We present a study of the textural signature of terrestrial weathering and related biological activity in the Tatahouine meteorite. Scanning and transmission electron microscopy images obtained on the weathered samples of the Tatahouine meteorite and surrounding soil show two types of bacteria-like forms lying on mineral surfaces: (1) rod-shaped forms (RSF) about 70–80 nm wide and ranging from 100 nm to 600 nm in length; (2) ovoid forms (OVF) with diameters between 70 and 300 nm. They look like single cells surrounded by a cell wall. Only Na, K, C, O and N with traces of P and S are observed in the bulk of these objects. The chemical analyses and electron diffraction patterns confirm that the RSF and OVF cannot be magnetite or other iron oxides, iron hydroxides, silicates or carbonates. The sizes of the RSF and OVF are below those commonly observed for bacteria but are very similar to some bacteria-like forms described in the Martian meteorite ALH84001. All the previous observations strongly suggest that they are bacteria or their remnants. This conclusion is further supported by microbiological experiments in which pleomorphic bacteria with morphology similar to the OVF and RSF objects are obtained from biological culture of the soil surrounding the meteorite pieces. The present results show that bacteriomorphs of diameter less than 100 nm may in fact represent real bacteria or their remnants.

Keywords: meteorites; bacteria; alteration; carbonates; exobiology

1. Introduction

The search for small life forms in terrestrial and extraterrestrial rocks has been prompted by the report of putative bacterial remnants in the Martian meteorite ALH84001 [1] and the description

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of small (less than 1 μm) bacteria-like objects in various sedimentary and volcanic rocks [2–6]. The debate has now moved to more general questions. What is the volume limit required for survival of an autonomous living organism? What are the criteria for assessing the presence of nanometer-sized remnants of life forms in rocks from the Earth, meteorites and Mars? We present new observations on the Tatahouine meteorite as well as microbiological experiments which address these two important issues. The previously described rod-shaped objects (60–80 nm in diameter and 100–600 nm in length) [7] on the surface of the Tatahouine meteorite minerals are among the smallest bacterial remnants ever characterized.

The proposition that rod-shaped objects (a few tens of nanometers in diameter and a few hundreds of nanometers in length) are remnants of former Martian living organisms in the ALH84001 meteorite is highly controversial [1,8–10]. Two types of objections have been raised against this claim. First, it has been argued that these objects are artifacts related either to sample preparation for electron microscopy or to mineralogical imaging artifacts [1,8–10]. The second objection comes from biological considerations and raises fundamental issues. A consensus has recently emerged that cells with a volume less than that of a 200 nm diameter sphere should not be viable [11,12]. This conclusion is valid for known life forms. However, such a conclusion is not obvious for fossils or dormant bacteria. In fact, there is no necessary simple relation between the size and shape of a living bacterium and that of its fossilized forms. Moreover, bacteria observed in situ are often smaller than those observed in pure culture (rich medium). Until now, the identification of nanobacteria in rocks has been based solely on morphological similarities to living bacteria, the only difference being the size of the organisms (less than 150 nm in diameter). Some bacteriomorphic objects can also result from non-biological processes [13]. New observations and microbiological experiments demonstrate that nanobacterial forms observed on the weathering products of the Tatahouine meteorite are indeed real bacteria. They throw light on the interpretation of remnants of life in rocks and the existence in Earth's rocks of unusually small viable bacteria or their remnants [12].

2. Summary of previous observations on the Tatahouine meteorite

The fall of the Tatahouine meteorite was observed in 1931 [14] and many samples were collected soon after and sent to the Museum d'Histoire Naturelle in Paris. The strewnfield was revisited in 1994 and several weathered fragments of the stone were recovered by sifting the first few centimeters of the soil. In previous studies, we reported the results of a scanning electron microscopic (SEM) investigation of pristine and terrestrially weathered samples of this meteorite [7,15]. High magnification ($>20\,000\times$) SEM images have revealed numerous bacteria-like forms on the surfaces of the meteorite minerals (pyroxene and chromite), and also on the secondary calcite crystals resulting from terrestrial weathering and mobilization by fluid circulation of carbonates from the soil to the fractures of the meteorite. It was suggested that these features correspond to mineralized casts of unusually small (nano) bacteria. In some fractures the carbonates and the associated bacteria-like objects form a rosette texture (diameter 80–150 μm, 15–20 μm thick) mimicking small bacterial colonies. The rosettes are systematically associated with saucer-shaped cavities in the orthopyroxene. They mainly consist of euhedral calcite crystals forming a rather porous aggregate. Similar bacteria-like structures in ALH84001 [1], in the Columbia River basalt [3,6] and in sediments [2,4,5] have been interpreted in terms of bacterial cells. In order to confirm our former suggestion and demonstrate that the features observed on the Tatahouine meteorite are bacterial remnants, we performed new microscopic observations using both scanning and transmission electron microscopies (SEM and TEM) as well as bacterial isolation from the soil in which the meteorite pieces were embedded and which invaded the fractures of the meteorite.

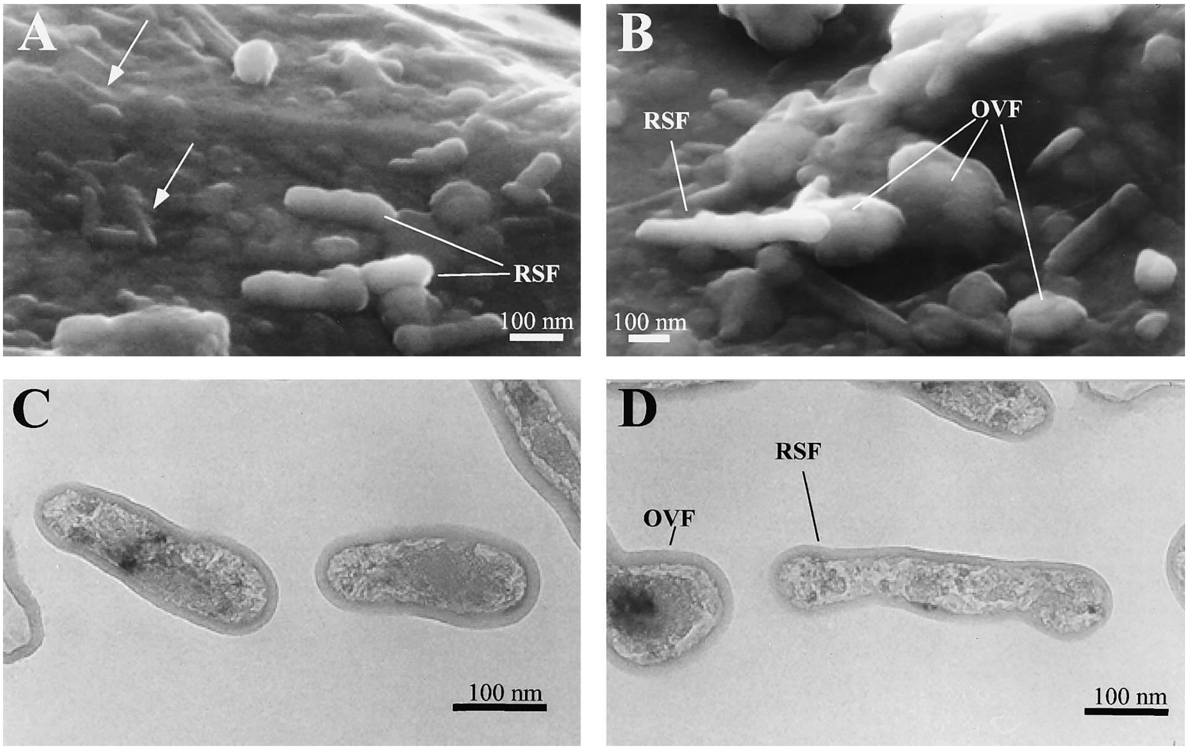

Fig. 1. SEM and TEM images of bacterial-like cells from the soil surrounding the pieces of the Tatahouine meteorite or filling its fractures. (A, B) High-resolution FEG-SEM images. Rod-shaped (RSF) and ovoid (OVF) forms lying on a crystallographic face of a pyroxene crystal. The size distribution of the RSF and OVF observed on this view is representative for all the samples examined. Note that some RSF objects have a diameter less than 60 nm and a length less than 200 nm. Some RSF and OVF are encrusted within the minerals (arrows). (C, D) TEM images of RSF and OVF. Corresponding EDS microanalyses are shown in Fig. 2. The bacteriomorphs look like single cells surrounded by a thick wall. The dark spots within the cells are diffracting nanocrystallites of NaCl and KCl as confirmed by EDS analysis and electron diffraction patterns (see Fig. 2).

3. Scanning and transmission electron microscopy observations

The SEM observations were carried out with a FEG-SEM Jeol JSM6301-F microscope. Samples were mounted on a carbon conductive adhesive tape followed by carbon coating with a Baltec modular high-vacuum coating system MED020. Operating conditions were 5–11 kV with a sample-to-objective working distance of 5–15 mm. TEM observations were carried out with Jeol 2000 EX and Philips CM30 transmission microscopes, operated at 200 kV and 300 kV, respectively. EDS chemical microanalyses were performed with a Ge detector allowing detection of C, N and O in scanning TEM mode (probe size of 8 nm). For the TEM observations, weathering products filling the fractures or surrounding the pieces of the meteorite were dispersed in a droplet of pure sterile water and deposited on carbon-coated copper grids and then evaporated.

From the SEM images obtained on the weathered samples of the Tatahouine meteorite and surrounding soil, two types of objects with bacteria-like forms can be distinguished. The first type is represented by rod-shaped forms (RSF) about 70–80 nm wide and ranging from 100 nm to 600 nm in length (Fig. 1), some of them appear segmented and their ends are rounded. The second type consists of more or less ovoid forms (OVF) with diameters between 70 and 300 nm. A closer look reveals that RSF and OVF are also embedded within the calcite or pyroxene crystals (Fig. 1). In this case, their sizes are in general smaller

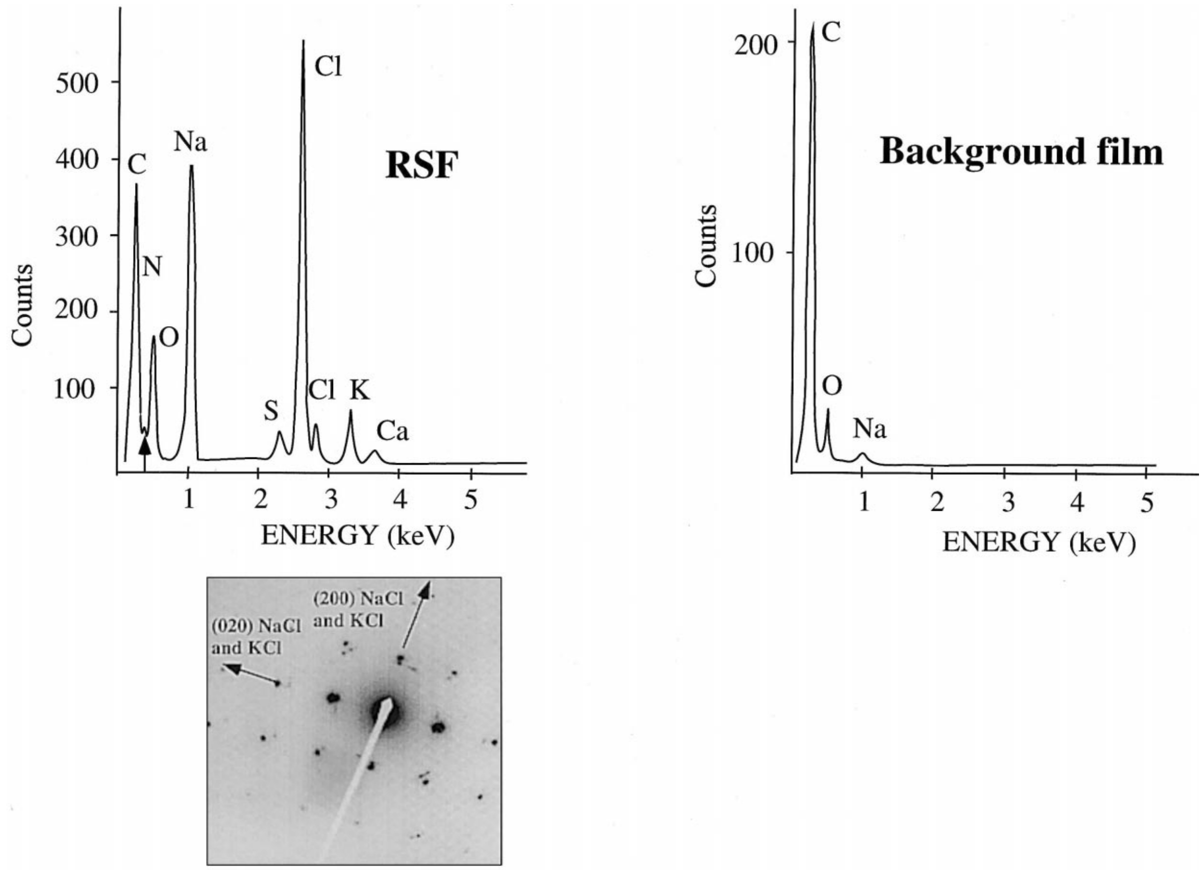

Fig. 2. Typical EDS and electron diffraction patterns obtained on RSF and OVF of the Tatahouine meteorite. (A) EDS spectrum showing the background signal of the carbon-coated copper grid on which the OVF and RSF have been deposited. Only C and small amounts of O are observed. (B) EDS spectrum obtained on a RSF object. C, O, N, Na, K, Cl, Ca and S are present in the bulk of the bacteriomorphs. No other element has been evidenced. (C) The strong Na, K and Cl peaks observed on the EDS spectra are related to the presence of small nanocrystallites of NaCl and KCl as shown by diffraction patterns. Beside these crystallites, the bulk of the cells do not diffract electrons in agreement with an amorphous content.

than those of the forms lying on a crystal surface. No other types of bacteria-like forms have been observed on the numerous samples examined so far.

TEM observations of the RSF and OVF further confirm their resemblance to bacterial cells. They look like single cells surrounded by a cell wall. Segmentation features are observed and in some cases, the RSF and OVF are related to each other. Nanocrystallites of NaCl and KCl were often observed within the objects as demonstrated by electron diffraction patterns and EDS chemical analysis (Fig. 2). Superimposed on the strong Na, Cl and K peaks, peaks due to C, O and N with traces of P and S are observed in the bulk of the objects. All other elements including Si, Al and Fe were below detection limits. In some places the bulk of the RSF and OVF does not diffract electrons in agreement with a non-crystalline content. The chemical analyses and electron diffraction patterns confirm that the RSF and OVF cannot be magnetite or other iron oxides, iron hydroxides, silicates or carbonates. The RSF and OVF are not simple inorganic weathering products usually observed in meteorites or soils. Their shapes are similar to those of bacteria but their sizes are below those commonly observed. All the previous observations strongly suggest that they are bacte-

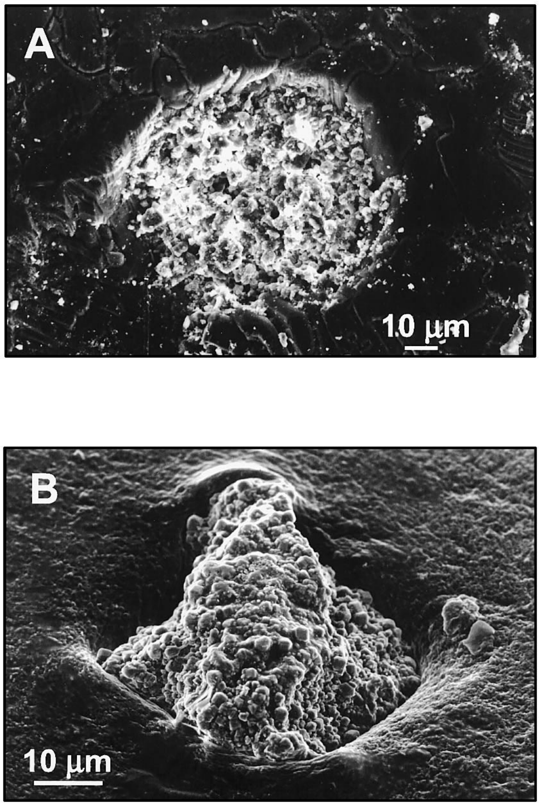

Fig. 3. Comparison between a calcite rosette of the Tatahouine meteorite (A) and calcite clusters precipitated by bacterial strain TTB 310 during its growth on an agar culture medium (B). The sizes of both colonies are similar, of the order of 100 μm. The grain sizes of the calcite crystals (~1 μm) are equivalent in both cases. All these similarities suggest that the carbonate rosettes observed within the fractures of the Tatahouine meteorite represent single bacterial colonies.

ria or their remnants. This conclusion is further supported by microbiological experiments.

4. Microbiological experiments

The following procedure was used for biological cultures. 4.3 g of soil surrounding the pieces of the meteorite was dispersed in 10 ml of demineralized sterile water and kept for 11 days at 4°C. 100 ml of this solution was diluted in 100 ml 10-fold diluted tryptic soy broth (TSB/10, Difco) supplemented with 100 mM $CaCO_3$. After 2 days incubation at 30°C, the resulting culture was filtered using a 0.45 μm pore diameter filter (Millipore). Twenty-four tubes containing 100 ml of the resulting filtrate and 900 μl TSB/10 were prepared and kept for 5 days at 30°C. Rod-shaped bacteria having diameters between 0.1 and 0.2 μm and ovoid bacteria with diameters of the order of 0.5 μm were observed by optical microscopy in eight of the 24 tubes. The contents of these positive tubes were streaked on agar culture medium (TSB/10+agar 15 g/l+100 mM $CaCO_3$) and kept for 29 days at 30°C. After this incubation time, small yellowish bacterial colonies 100 μm in diameter were observed. They are very close in size and shape to the rosettes of carbonates observed within the meteorite fractures [7,15] (Fig. 3). They also form a porous aggregate of euhedral micrometer-sized calcite crystals associated with bacterial cells having morphologies very similar to the RSF and OVF observed on the weathered pieces of the meteorite and surrounding soil except that their sizes are two to three times larger (about 120–170 nm wide and 900–2000 nm in length).

After 10 purification steps consisting in successive streaking of single colonies, all purified colonies contained pleomorphic bacteria with these two morphotypes originating from the same bacterial cell (Fig. 4). Cell division was only observed with spheric forms (OVF). TEM observations show that their cell wall displays an electron-dense outer glycocalyx (Fig. 4). A double membrane is observed in agreement with a Gram-negative enzymatic reaction. Nanocrystallites of NaCl and KCl are found close to or within the wall of the bacteria growing in agar culture medium (Fig. 4). Serial replicates always gave the same microcolonies containing both morphotypes. The 16S rDNA of the bacterial strain TTB 310 containing both OVF and RSF morphotypes was sequenced and only one DNA sequence was observed, demonstrating that both morphotypes represent the same bacterium. This sequence has been deposited in the GenBank database (accession number AF144383). This bacterial strain belongs to a new genus to be described. The closest described genus is *Acidovorax* (β-Proteobacteria). All intermediate stages between OVF and RSF individuals are observed by TEM (Fig. 4).

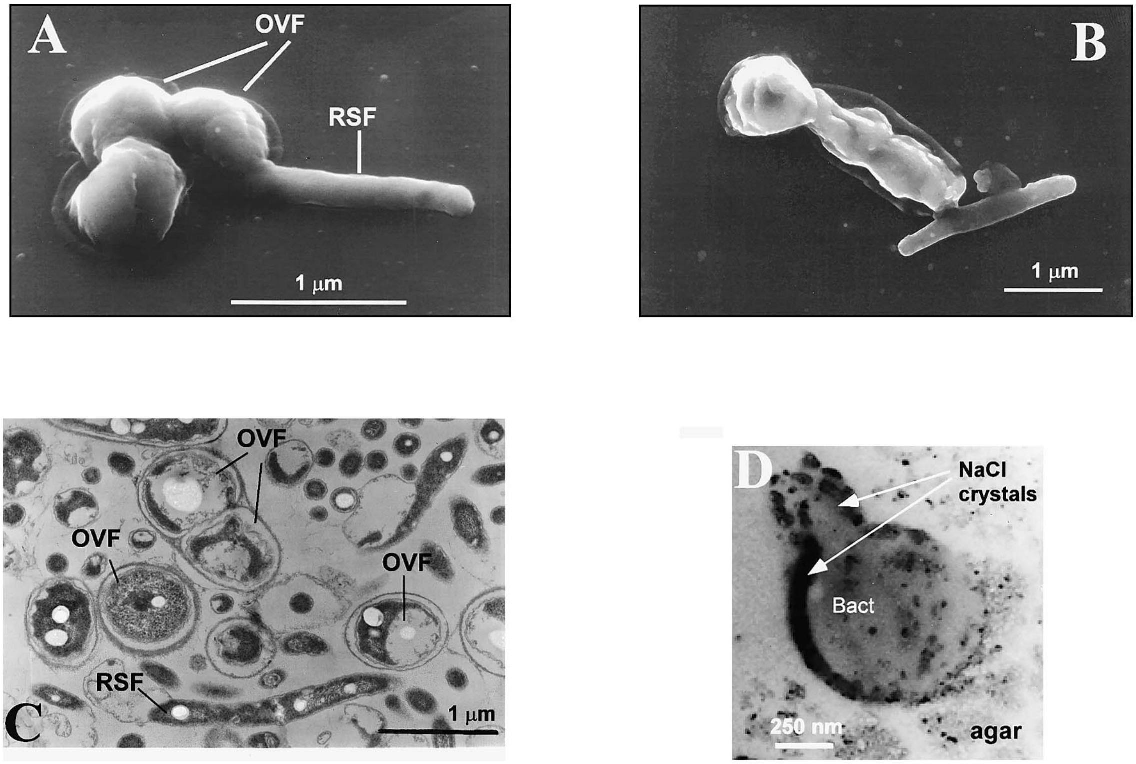

Fig. 4. SEM and TEM images of the pleomorphic bacterial strain TTB 310 grown after biological culture of the weathering products of the Tatahouine meteorite and surrounding soil. (A, B) SEM images showing the close association of elongated and ovoid bacterial cells. The bacteria were pelleted by centrifugation and dispersed on a glass slide. They are similar to the RSF and OVF forms observed on the surface of the minerals of the meteorite (compare with Fig. 1). They have sizes two to three times larger than those of the RSF and OVF forms. (C) TEM micrograph of ultra-thin sections of the bacteria showing the cellular content and the double membrane. Note also the various intermediate stages between OVF and RSF forms. (D) TEM micrograph of bacterial cells. The sample was obtained by scraping with a carbon-coated TEM grid the surface of a bacterial colony similar to that shown in Fig. 3B. The bacterial cells are surrounded by small crystals of NaCl and KCl probably incrusted in the cell wall.

5. Discussion

From the TEM and SEM observations and results of the bacterial culture we conclude that the RSF and OVF observed in both soil and weathered meteorite samples are bacteria or at least remains of bacteria. Some of them are viable and lead to the growth of bacterial colonies when cultured on biological media. Pleomorphic bacteria are not very usual and observing such similar pleomorphic-like forms on the meteorite minerals and in the biological cultures is striking. Moreover, the cultured bacteria precipitate clusters of calcite similar to those observed on the altered meteorite pieces.

The factor 2–3 size difference between the bacterial cells grown in pure culture and those observed on the meteorite samples and surrounding soil has to be explained. Bacterial cells grown on culture media rich in nutrients are always larger (up to a factor 4 in diameter) than those grown in limiting conditions which will increase their generation time [14]. It is thus expected that they have a larger size than the one they would adopt when stressed for instance by a nutrient-poor environment.

The volumes of the bacterial cells in pure culture lie between 1×10^{-2} and 4.5×10^{-2} μm^3, i.e. those of spheres with radii between 0.15 and 0.25 μm. These sizes are very comparable to those of organisms called 'nanobacteria' (about 0.25 μm in diameter) isolated from human and bovine blood [16]. The RSF and OVF in the Tatahouine meteorite have volumes ranging between 1×10^{-3} and 6×10^{-3} μm^3, corresponding to the volumes of spheres with radii between 0.06 and 0.11 μm. These latter volumes are equal to or smaller than the size cut-off proposed at an American National Academy of Sciences workshop [11] during which it was concluded that the most basic molecular machinery of life (DNA+ribosomes+usual cellular content) to be efficient and self-reproducing needs a minimal volume corresponding to that of a 0.2 μm diameter sphere.

The present observations have important implications for the search for past life on both Earth and extraterrestrial objects. They show that bacteriomorphs of diameter less than 100 nm can in fact represent real bacteria or their remnants. They also permit the study of the textural signature of biological activity in rocks (carbonate rosettes). Small bacterial remnants or their appendages with sizes down to 50 nm in diameter and 200 nm in length, encrusted in minerals from the Tatahouine meteorite, represent the smallest fossils so far described. Some of the 'nano-objects' described in the ALH84001 meteorite have sizes very comparable to those observed in the Tatahouine meteorite (i.e. 200–600 nm in length and 40–60 nm in diameter). Without inferring their biological origin, the size argument used against the presence of putative life remnants (of Martian or terrestrial origin) in the ALH84001 meteorite is no longer valid for these objects.

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