

## A Presumptive Fossilized Bacterial Biofilm Occurring in a Commercially Sourced Mars Meteorite

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### Abstract

A commercially sourced Mars meteorite sample was found to contain a presumptive fossilized bacterial biofilm composed of a wide variety of bacteria-like morphologies. We conclude this presumptive biofilm, and other biomorphs, provide preliminary evidence for the occurrence of fossilised bacteria in a Mars meteorite and therefore that life once existed on Mars.

**Keywords:** Martian meteorite; Microfossils; Panspermia

### Introduction

A number of papers have reported the presence of bacteria-like features in Martian and other meteorites [1-4]; the most famous of these being the claimed nanobacteria-like, fossilized structures found in the Allen Hills (ALH 84001) meteorite [5]. To date none of these claims has been fully substantiated, largely because meteorites contain mineral features which closely mimic bacteria and other microorganisms, making it difficult to distinguish between potential fossilized bacteria and non-biological artefacts [6]. The aim of the study reported here was to use scanning electron microscopy (SEM) to examine a sample of a commercial sourced Martian meteorite of known provenance to look for bacteria-like structures and where found, to provide evidence to show that such bacteriamorphs are not mineral artefacts.

### Materials and Methods

#### Details of the meteorite examined

The single sample used here was cut from a larger meteorite sample, listed as Northwest Africa 4925 NWA 4925; it was obtained from Meteorites for Sale.com. The authenticity of the sample is guaranteed by fellow members of the International Meteorite Collectors Association. It was found in Erfoud, Morocco, in 2007 and is classified as an achondrite, i.e. a Martian, olivine-phyric shergottite). One fragment partly covered by fusion crust weighing 282.3 g was found. The meteorite displays a porphyritic texture with large chemically zoned olivine megacrysts set into a fine-grained groundmass composed of pyroxene and maskelynite. Minor phases include chromite, sulphides, phosphates, and small Fe-rich olivines. The olivine megacrysts often contain melt inclusions and small chromites. Its mineral composition (EMPA) is: Olivine,  $\text{Fa}_{27.6-46.8}$ ; pyroxene,  $\text{Fs}_{20.0-37.7}\text{Wo}_{3-14.8}$ ; maskelynite,  $\text{An}_{67-69}$ . It is classified as an achondrite (Martian, olivine-phyric shergottite); severely shocked with some melt pockets; moderately weathered.

#### Sterilization and lysis of potential modern biofilms

A single meteorite sample was used as sent by the supplier and was not subjected to further cutting. It was immersed in 80 per cent ethanol for 1h, washed twice with sterile deionized water and transferred to a sterile plastic petri dish before being examined under the SEM. All water used was sterilized by autoclaving at 120°C for 20 min. and then filtered through a 0.1 micron micropore filter (Nalgene).

### Scanning electron microscopy

The meteorite sample was placed in a staging chamber with the face to be analysed flush to the base. Konductomet phenolic mounting compound (20-3375-016) was used to stage the sample. Usually surface grinding and then polishing of the sample surface is undertaken at this point however in this case only an instantaneous grinding process was done. This was done to remove any build up that might be present atop the surface to be studied and ensure that only fresh sample material be exposed. The coarseness of pile used was 120 microns using a Bueler Automet 250 for 5 seconds with a touch force of 20N, a head speed of 50 RPM and a Platen speed of 140 RPM. A second sample was prepared presenting the outside surface of the 'meteorite'. This was staged atop a conductive carbon tab. Due to the relatively low conductive nature of the samples, in order to minimise charging effects and optimise image acquisition the sample was coated using an Emscope gold sputter coater. The sample was coated for a deposition-duration of 1 minute at 15 milliamps. Ahead of introduction into the SEM the samples were placed in a vacuum chamber overnight to remove any remaining moisture from the porous sample. No chemicals or concentrated alcohols were introduced at any stage as a cleaning step. The samples were all delicately irrigated using de-ionised water.

### Results and Discussion

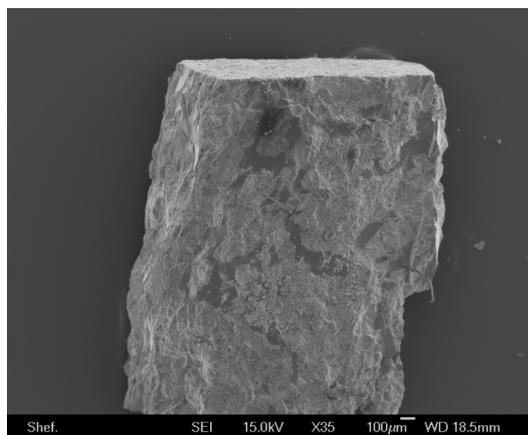
Figure 1 shows the meteorite sample under the scanning electron microscope (SEM); the sample has clearly been cut from a larger piece, so that the exterior surfaces would originally have been inside the meteorite. The surface of the sample was carefully scanned and imaged. Figure 2 shows one of two areas of the surface showing the presence of a presumptive bacterial biofilm consisting of a number of forms which look remarkably like bacteria and which had they been observed on the

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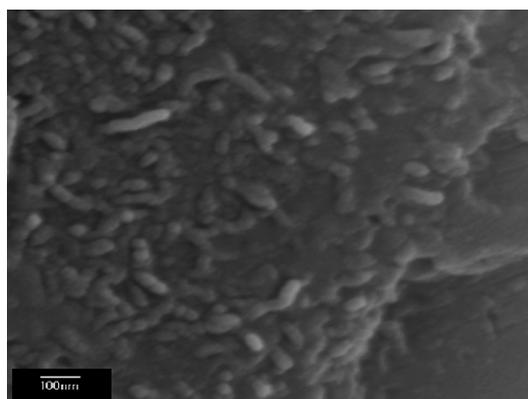
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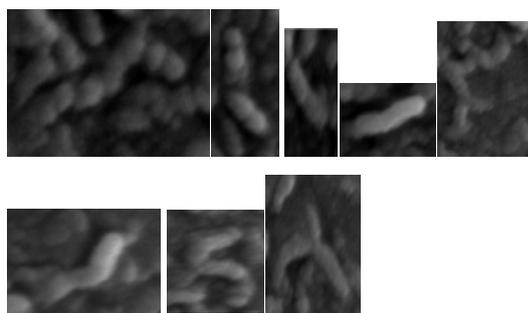
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**Figure 1:** The Meteorite sample. The images shown below were taken from the main face which, before cutting, would have been inside the meteorite sample.



**Figure 2:** The presumptive Martian bacterial biofilm showing cocci, rods and other bacteria-like morphologies (bar represents 100 nm).



**Figure 3:** Details of the presumptive bacteria-like found in the Mars meteorite biofilm showing various bacteria-like forms and groupings (Figure 2 for dimensions).

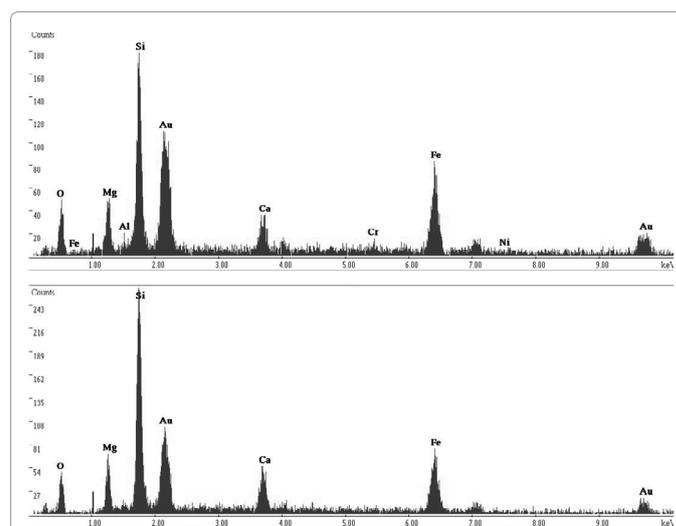
surface of a terrestrial soil or rock sample would be regarded by most microbiologist as comprising a distinct bacterial biofilm. Details of the individual bacterial types are shown in Figure 3 which apparent rods, cocci, spiral shapes bacteria-like chains, and individual rods occurring in star-shaped and more complex groupings. The chemical composition of both the presumptive biofilm area and an adjacent non-biofilm region, as determined by EDX, is shown to be essentially identical and

being made up principally of silicon iron, magnesium, calcium and oxygen, i.e. a composition typical of a meteorite.

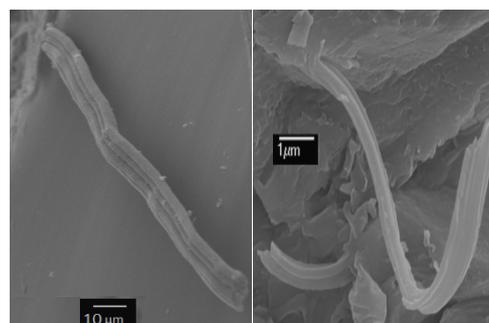
We suggest that (Figures 2 and 3) shows a fossilized bacterial biofilm made up of typical bacterial forms, which would have originally been located inside the original meteorite from which this sample was cut. It could be argued that the image is of a) an Earth-derived biofilm which fossilized after the meteorite had landed or b) a modern biofilm made up of living bacteria. The fact that the bacteria show no signs of lysis, after having been exposed to 80% ethanol suggests that this is not the case. The possibility that a modern terrestrial biofilm might have undergone mineralization during the period when the meteorite resided on Earth, seems highly unlikely. Similarly it is highly unlikely that a modern terrestrial biofilm could have formed from an air-derived bacterial inoculum, while, the meteorite was kept in storage.

EDX analysis (Figure 4) shows that the presumptive biofilm region is mineralized and has essentially the same chemical composition of an adjacent area of the meteorite which is free of the presumptive biofilm. These findings, we maintain, confirm, that the presumptive biofilm is part of the Mars meteorite and is not a modern biofilm made up of contaminating terrestrial bacteria.

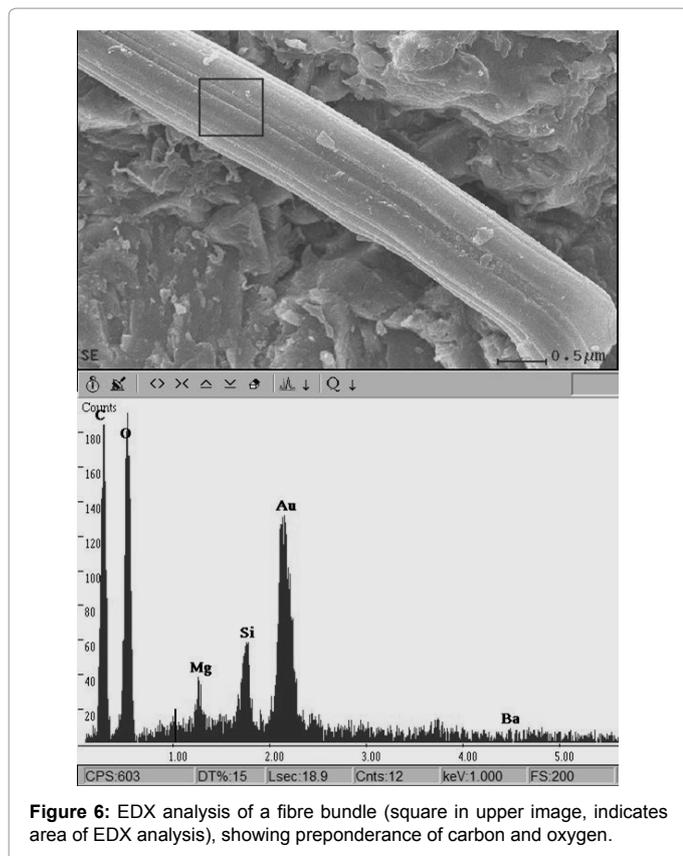
It could also be argued that the bacteria-like structures seen here are



**Figure 4:** EDX of A, the "biofilm" region and B an area of the meteorite away from the "biofilm".



**Figure 5:** Filaments on surface of the Mars meteorite, A, a filament bundle showing folding at the top (A); B, Attachment of filament bundle to the body of the meteorite (b).



**Figure 6:** EDX analysis of a fibre bundle (square in upper image, indicates area of EDX analysis), showing preponderance of carbon and oxygen.

mineral artefacts which simulate bacteria. Our response would be that, if this were the case, it would be a remarkable coincidence that such a wide variety of apparent bacterial forms would appear so closely packed into an apparent biofilm. Unlike the claimed bacterial form seen in the Allen Hills meteorite, the bacteria-like forms seen here are not nano-sized but, at around 0.2 micron, are similar in size to terrestrial bacteria seen growing in naturally occurring, nutrient-limited environments found on Earth [5]. Again, unlike the Allen Hills form, the putative bacteria found here are large enough to avoid arguments over whether or not they could contain a complete bacterial genome.

A final argument against the bacteria-like forms, seen here being, Mars-derived bacteria is that they appear *too much* like terrestrial bacteria. However, this argument is only valid if it is assumed that bacteria from a non-terrestrial source would, of necessity, differ in morphology from those found on Earth. If, as we think likely, both Earth and Mars derived their bacteria from a common source, this criticism will not hold [7].

Figure 5 shows another presumptive biomorph present in the meteorite under study, occurring in the shape of bundles of long fibres which are folded back on themselves and are attached to the body of the meteorite. Analysis of a fibre bundle by EDX (Figure 6) shows a preponderance of carbon and oxygen, with smaller amounts of silicon and magnesium, a composition which is suggestive of a mineralized biological entity. The fact that the bundles are attached to the body of the meteorite shows that the bundles are not contaminating fibres of natural origin (e.g. cotton), nor man-made fibres. In terms of morphology the bundles appear to be made up of filamentous microbes, possibly fungi or filamentous algae or cyanobacteria. The filaments are analogous to

similar filamentous structures claimed by Hoover [3] to occur in the Orgueil carbonaceous meteorite, which the author claims are fossilized cyanobacterial filaments of Mars origin.

The nanobacteria-like features found in the Allen Hills meteorite have not yet been finally confirmed to be fossilized Martian bacteria [6], this despite the fact that this meteorite is possibly the most intensively studied of all geological samples. Final confirmation that the biofilm and filaments described here are biological and originate from Mars will presumably be likewise very difficult to achieve [8]. For the moment then, we can only present what we claim is provisional evidence for the presence of a fossilized bacterial biofilm on the inside cut surface of a Mars meteorite sample. Since biofilms form in environments rich in water, we suggest that the presumptive bacterial biofilm, provided here (and the associated filament bundles), formed in a watery environment on Mars. This conclusion appears consistent with recent findings from the NASA Mars Curiosity Rover of fine-grained sedimentary rocks which were interpreted to represent an ancient lake preserving evidence of an environment that would have been suited to support life [9]. It is also relevant to note that a recent re-evaluation of the results from the Labelled Release Experiment on the Mars-Viking probe of 1976 has concluded that extant Martian surface microbiology was most likely to have been discovered at this time [10].

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