IDENTIFYING FOSSIL BACTERIA IN MARTIAN MATERIALS. F. Westall, D. S. McKay, and E. K. Gibson Jr., Mail Code SN2, NASA Johnson Space Center, Houston TX 77058, USA.

Within the next decade robotic missions are going to Mars with the search for evidence for extant and extinct life as at least one of the mission objectives. Moreover, the first Martian samples will be returned to Earth in 2008. It is, thus, imperative that we can be certain that we can identify life in Martian rocks [1]. In this paper we will not be discussing extant life but will concentrate on fossil life.

The kind of life that we can expect as fossils on Mars is likely to have been relatively simple. The rationale for this hypothesis is as follows. Environmental conditions on Mars apparently deteriorated early in the history of the planet (ca. 3.5 Ga [2]). At this period on Earth the only life forms appear to have been simple prokaryotes [3, 4], with the appearance of oxygenic photosynthesising cyanobacteria probably in the Late Archean period (3.0 - 2.5 Ga [5]). Increases in atmospheric O_2 above 1-2% present atmospheric level (PAL), considered to be essential for the evolution of photosynthesising eukaryotes [6], first occurred in the 2.2-1.9 Ga interval [7]. The earliest eukaryotes identified occur in rocks about 2.1 Ga in age [8]. Thus, at least one billion years seems to have been needed for the evolution of oxygenic photosynthesis on Earth, and two billion years were necessary for the evolution of more sophisticated organisms. Furthermore, this evolution took place in more or less optimal conditions (give or take the odd planet-sterilising, meteorite impact [9]). Given the relatively long time period for initial evolution on Earth it, thus, seems unlikely that anything more than simple prokaryotes could have evolved on Mars. In this paper we address the salient factors in identifying fossil bacteria and address some of the pitfalls involved.

Important criteria in the identification of fossil bacteria

(1) *Suitable rock type (environment of deposition):* Most searches for Martian landing sites with exobiological potential concentrate on areas where there has obviously been water, e.g. lake-filled craters, for the simple reason that life, as we know it, needs water. Terrestrial prokaryotes, however, are remarkably resilient and are being found in the most unlikely, extreme environments [10-13], as well as in association with non-sedimentary rocks at depth [14].

(2) *Size:* More than 90% of bacteria fall within the 0.5-2 micrometer size range. Some can be much larger, e.g. the cyanobacteria, whereas some can be smaller, e.g. myxoplasma. Submicrometer-sized “nannobacteria” have been described (and disputed) from the natural environment (15, 16).

(3) *Shape:* Bacteria vary widely in shape from round, oval, rod-shaped, curved, spiral to amorphous. Usually one species is characterised by a particular size and shape but some species are pleomorphic, i.e. show varying shape. Both size and shape can change depending upon the age of the colony of a particular species and its nutritional status.

(4) *Cell wall morphology or texture:* The bacterial cell wall may be smooth and turgid, covered with “excrescences (blobs of exopolymer), wrinkled (when dead or under osmotic stress), or deflated (when the dead organism has lysed and its body contents (cytoplasm) have escaped the cell wall).

(5) *Cell division:* Bacteria reproduce in characteristic ways, resulting in characteristic associations. Reproduction may be by cell division, with the resulting formation of simple to complex associations, e.g. pairs, chains, to three dimensional associations. Bacteria also reproduce by budding, as well as sexually (n.b. the latter is not a fossilisable characteristic).

(6) *Colony formation:* Bacteria, through their reproduction, form colonies of cells, usually with millions of individuals but also with fewer numbers in young colonies or stressed regimes.

(7) *Consortia:* In the natural environment most colonies are formed of consortia consisting of more than one species.

(8) *Association with biofilm:* Bacterial colonies are always associated with mucus or slime (exopolymeric substances, or EPS) of their own production.

(9) *Composition:* Bacteria are soft-bodied, carbon-based entities. During fossilisation the carbon may or may not be preserved. Known examples of fossil bacteria have been preserved as (a) carbonaceous compressions in soft sediments [17]; (b) permineralised fossils in which the originally organic structure is permeated by silica which fixes the organic molecules (in varying stages of degradation) [18]; (c) mineral replacement in which the organic structure acts as a template for mineral nucleation, after which it degrades and disappears, leaving an mineral cast and/or crust [4,19,20] (minerals replacing micoorganisms include silica, clays, oxides,

sulphides, carbonates and phosphates); and (d) empty moulds or impressions in soft sediments or minerals [4] (moulds may be filled with minerals at a later time).

The identification of fossil bacteria will be based on a combination of most of these features together. However, a number of abiological structures (bacteriomorphs) may be confused with fossil bacteria, as shown in the table below.

Criterium	Spherical bacteria	Spherical bacteriomorphs*
Size (diameter)	generally 0.5-2 microns	variable
Shape	spherical, oval to slightly irregular	spherical, oval to slightly irregular
Shape distribution	generally narrow	variable
Walls	smooth, rough, wrinkled, deflated	smooth or wrinkled
Cell division	1D, 2D, 3D	apparent cell division (juxtaposition of spheres)
Colonies	yes	apparent colonies
Consortia	yes	could occur with *bona fide* fossil bacteria
Water	occur with aqueous deposits	occur with aqueous deposits
Biofilm	yes	possible
Composition	organic	organic, mineral

*e.g. gas bubbles, organic micelles, mineral precipiates

Criterium	Rod-shaped bacteria	Rod-shaped bacteriomorphs#
Size (diameter)	generally 0.5-2 microns	variable
Shape	round cross section, round-ended, may be bent	generally angular, but can be amorphous
Shape distribution	generally narrow	variable
Walls	smooth, rough, wrinkled, deflated	smooth
Cell division	1D, 2D, 3D	apparent cell division (twinning)
Colonies	yes	apparent colonies
Consortia	yes	could occur with *bona fide* fossil bacteria
Water	occur with aqueous sediments	occur with aqueous sediments
Biofilm	yes	possible
Composition	organic	mineral

e.g. crystals

The many morphological similarities between fossil bacteria and abiogenic spheres and rods should not be a reason for precluding any search for morphological fossils since abiogenic structures generally do not show **ALL** the characteristic features of fossil bacteria. Additionally, the presence of abiogenic spheres and rods does not preclude the possibility that true microfossils may also be present in the same sample. Furthermore, it should be stressed, that it **IS** possible, in many cases, to identify fossil bacteria on the basis of morphology [3, 4,19,20], although, the interpretations may be further strengthened by additional data, such as the association of a carbon isotope signature indicative of bacteria fractionation or the presence of biogenic organic molecules.

In conclusion, it is advisable that anyone interested in looking for bacterial fossils be well grounded in the observation of both modern bacteria and crystal morphologies. Experience of this kind allows the researcher to understand associations and combinations of features in a colony of bacteria that would not be present in an association of abiogenic bacteriomorphs.

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