Fossilized Nanobacteriaforms from the Green River Formation: Ancient Life Form or Just a Product of Calcium Carbonate Precipitation?

Glenn M. Mason

Indiana University Southeast, Department of Geosciences, New Albany, Indiana 47150

Abstract

Controversy surrounding the existence of minute bacteriaforms called *nanobacteria*, *nannobacteria*, or *ultramicrobacteria* has existed since their discovery in 1988. These tiny bacteria-like forms, recognized in the 0.05 to 0.5 micrometer range have been disputed as living entities by some researchers because it is believed that they are too small to facilitate DNA replication. Samples of Green River Formation oil shale from Wyoming, long valued for its exquisite fossilization, revealed possible *nanobacteria* from the Tipton Shale Member associated with a fossilized bacterial mat. Mineral precipitates are generally observed as angular forms, with their shape reflecting the crystalline structure. These *nanobacteriaforms* clearly have a rounded or filamentous appearance, strongly suggestive of living forms, but with none of the telltale angularity normally observed with minerals. Associated with carbonate-rich sediments and other larger fossilized bacteria-like forms, these tiny particles may represent an important step in furthering our understanding of how a complex geochemical system, like the Eocene Lake Gosiute or Lake Uinta produced such a wide diversity of mineral forms. Climate-induced evaporation, contributions by volcaniclastic sources, as well as detrital material played a dominant role in the mineralogy of the Green River Formation. However, the biological contribution to mineral genesis should not be overlooked. Not that long ago, mineral precipitation was considered to be the exclusive product of inorganic reactions; now, the common association of fossil bacteria (and nanobacteria) may further demonstrate the complex intertwined and possibly inseparable relationship that exists between the living and geochemical worlds.

Background and Geological Setting

The lacustrine sediments of the Green River Formation (GRF) in Colorado, Wyoming, and Utah have been the object of intense study for more than a hundred years. A unique assemblage of minerals, some identified nowhere else, characterizes the GRF. In addition to minerals, the GRF has produced one of the most remarkable fossil assemblages known, consisting of vertebrates, invertebrates, microorganisms, and plants, ranging from microscopic bacteria to 16 ft. long crocodiles.

A great deal of information exists concerning these lacustrine sediments. Roelher (1993) described the stratigraphy of Wyoming's GRF, pointing out that the saline minerals occurred primarily in the Wilkins Peak Member and were absent from the oil shale of the underlying Tipton Member and the overlying Laney Member.

In Wyoming, the GRF was deposited during a four million year interval during the Eocene as Lake Gosiute, which occupied parts of the present-day Green River, Washakie, Sand Wash, and Great Divide Basins covering an area of approximately 16,000 mi^2 (43,500 km^2) in southwestern Wyoming and adjoining parts of Utah and Colorado. During its life, the lake passed through three major stages, each of which corresponds to a member of the formation. From oldest to youngest, are:

1) the Tipton Member, which consists of oil shale and scattered dolomitic mudstone deposited over ~1 million years when the waters of Lake Gosiute were fresh;

2) the Wilkins Peak Member, which consists of oil shale, marlstone, limestone, and evaporite minerals with beds of sandstone, siltstone, volcanic tuff, and mudstone deposited over ~1 million years when the climate became more arid and evaporation exceeded the supply of water, which resulted in the deposition of evaporitic and saline minerals; and

3) the Laney Member which consists of oil shale, marlstone, fine-grained sandstone, and minor beds of limestone and altered tuff deposited over ~2 million years when Lake Gosiute achieved its greatest expansion and the lake changed from a hydrologically closed basin, with hypersaline lakes and playas, to a fresh water hydrologically open basin (Carroll and Bohacs, 1999). Volcanic tuffs have been used by Rhodes et al. (2002) to date the Green River sequence to approximately 52 - 48 Ma.

Sample Source and Methods

Samples for this work are from the U. S. Energy Research and Development Administration/Laramie Energy Research Center (ERDA/LETC), Black's Fork Core Hole No.1, located in SE1/4 of the NE1/4 of sec. 24, T16 N, R 108 W, Sweetwater County, Wyoming drilled in 1976. The Black's Fork Core Hole No. 1 was cored from a depth of 181.0 to bottom of hole at 1676.6 feet, with the borehole terminating in the Wasatch Formation. Figure 1 shows the approximate location of the sample area and lithology and stratigraphy of the GRF.

Sedimentary rocks of the GRF, except for clastic units and some tuffaceous horizons, are too fine-grained to be studied effectively by standard optical techniques. The average particle size of GRF sediments has been found to be in the 5 micrometer particle size (Tissot and Murphy, 1960). As more than 90% of bacteria fall within the 0.5 to 2 micrometer size range, Scanning Electron Microscopy (SEM) was used to investigate the sedimentary rocks. The original observation of bacteriamorphs in GRF samples occurred by accident as the author was examining samples for mineral relationships unrelated to bacterial morphology.

To Nanno or Not to Nanno ... that is the question!

First observed in 1981, then published by Morita (1988) as *ultramicrobacteria*, very small forms smaller than 300 nm; the term "*nanobacteria*" was coined. Folk (1992) identified structures he called *nannobacteria* (written with double "n") in travertine from the hot springs of Viterbo, Italy. A convention has been adopted between researchers to name the nanoparticles isolated

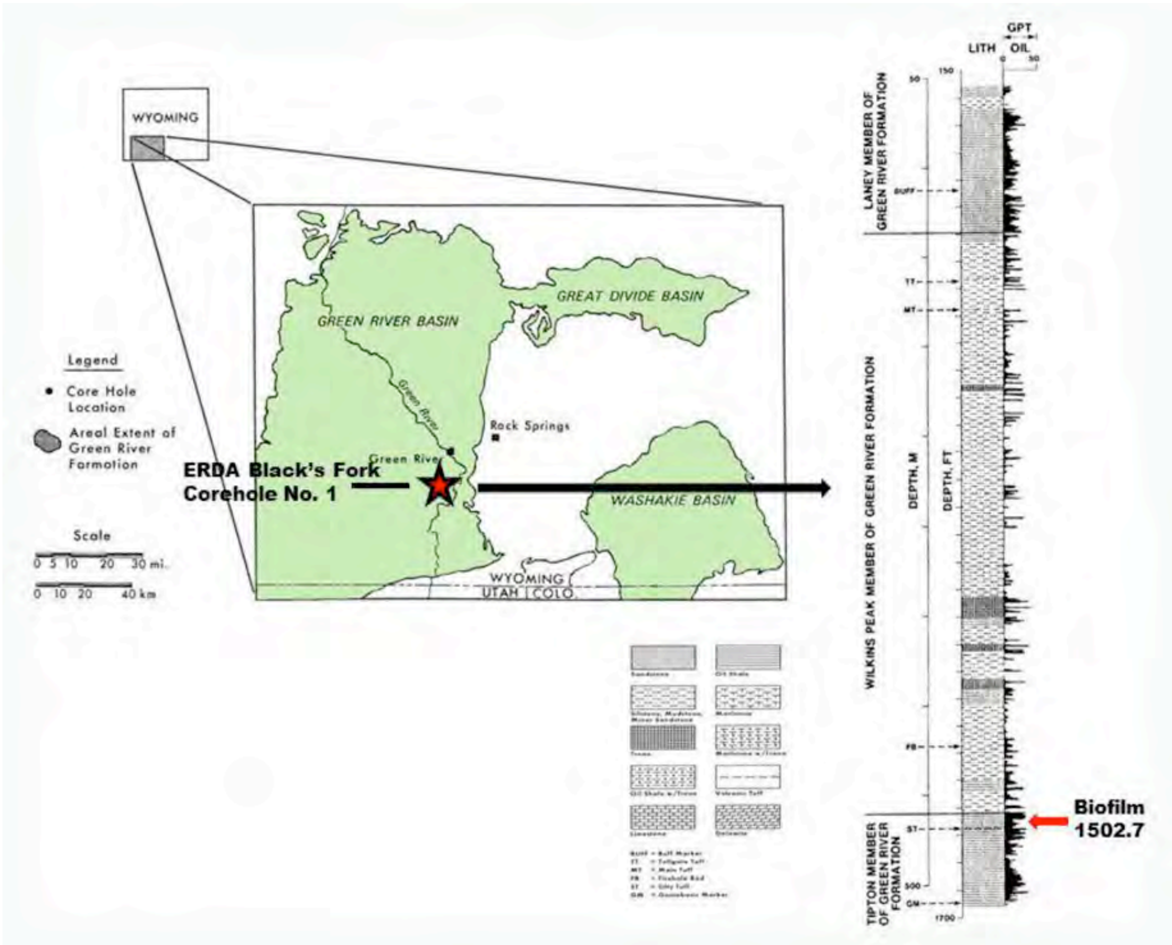

Figure 1. Approximate location of sample area with stratigraphic and lithologic column of U.S. ERDA Black's Fork shown.

from geological specimens as _nanno-bacteria_, and those from biological specimens as _nanobacteria_. Using an SEM, Folk observed extremely small objects that appeared to be biological in the travertine. He proposed that *nanobacteria* are the principal agents of precipitation of all minerals and crystals on Earth formed in liquid water and that they also cause oxidation of metals.

Controversy surrounding the existence of minute bacteria forms called *nano-bacteria*, *nannobacteria*, or *ultramicro-bacteria* has existed since their discovery. These tiny bacteria-like forms, recognized in the 0.05 to 0.5 micrometer range, have been disputed as living entities by some researchers because it is believed that they are too small to facilitate DNA replication. Few examples have been reported of bacterial cells less than 200 nm in diameter, which is the common lower size limit recognized by textbooks of microbiology. In general, many researchers in the biological community concur that nanobacteria are too small to contain the essential building blocks of living things.

Enthusiasm for the existence of nano-bacteria was boosted with the discovery of the Martian meteorite ALH84001 from Antarctica (McKay et al. 1996). Bacteria like-forms in the 0.1 micrometer range, exactly resembling the nannobacteria found on Earth were discovered in this meteorite. If nanobacteria are living organisms, their significance to terrestrial processes may be profound. Nannobacteria may be mediating many processes currently assumed to be controlled by inorganic chemical reactions, such as low-temperature precipitation of dolomite, oxidation of iron, and the formation of clay minerals on the Earth's surface (Folk, 1993)

Additionally, some researchers still feel that nanobacteria may also be controlling processes within organisms such as formation of shells, bones, teeth, calculus, and arterial plaque. Nanobacteria have been reported from bovine, rabbit and human blood and may be associated with or contributing to numerous human diseases associated with mineralized amyloid deposits in human tissue including inflammatory bowel disease, Alzheimer's disease, and Crohn's disease among others.

The most important scientific battleground is over the nano-bacteria sometimes called calcifying nanoparticles (CNP) that have been found in humans. For the medical profession, the important question is not whether they are living things, but whether they really do cause certain diseases and how they can best be treated. The observation of nannobacteria structures in the Eocene Age sediments of the GRF illustrate the widespread occurrence in both time and space of these remarkable small forms.

Fossil Bacteria–like Forms

Mason, (2005) identified five distinct *bacteriamorphs* (so called because they were identified by morphology alone - no internal cellular structure is visible and DNA testing is not possible due to fossilization) in samples from the U. S. ERDA/LETC, Black's Fork Core Hole No.1. It was later determined that the bacteria-like forms were part of a series of layers of sediment that represented several fossilized bacterial films from the Tipton Member of the GRF. Environmental, sedimentological, and chemical conditions must have been precisely in tune for the degree of preservation observed in the sedimentary rocks.

The first bacteriamorphs to be observed were the rod or rice-grain shaped forms approximately 10 µm long and 1 µm in width as shown in Figure 2. Entire layers (thousands of individual forms) of these bacteriamorphs were found and bear an uncanny resemblance to modern bacteria, although completely fossilized by mineral replacement with calcite ($CaCO_3$) and commonly coated by smectite (likely montmorillonite, a hydrous Na-Ca sheet silicate - Mason, 2005). Because the internal structure of the fossil bacteriamorphs was not observable, positive identification is not possible.

Nannobacteria

In addition to the bacteria-like forms, which had plausible modern counterparts, additional forms were observed in the same fossilized bacteria films. At the time of the initial observation, little attention was paid to these forms because of their extreme small size, because they did not fit the criteria set by Westall (1999) for consideration as bacteria-like forms. It was not until a chance discussion with Dr. Robert Folk

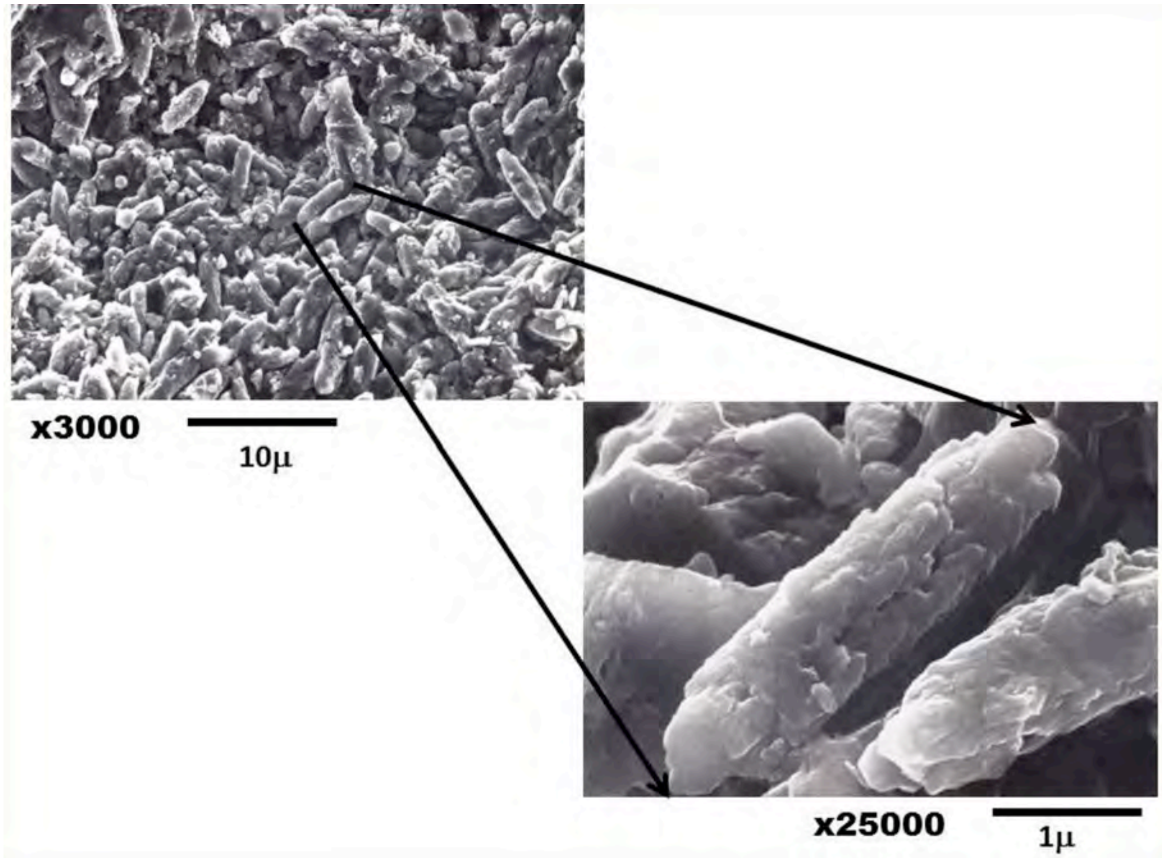

Figure 2. SEM photograph of original rod-shaped fossil bacteriamorphs identified from U.S. ERDA Blacks Fork core hole No. 1. Fossilized bacterial film from 1502.7 feet.

that the possibility that these were *nannobacteria* occurred.

Further observation of the fossilized bacterial films revealed the possibility of numerous additional bacteria-morphs, although far smaller than the originally observed rod-like forms. Figure 3 is a SEM photograph of numerous spherical to slightly oval *nanno-bacteria* forms less than 500 nm in length, with many less than 100 nm observable. Whereas the original rod shaped bacteriamorphs were observed to have a distinct smectite-like clay coating (see Figure 2) the smaller forms did not clearly have the coating or it was beyond the resolution capabilities of the instrument. It could not be determined if the smaller forms, therefore, were different in origin or composition from the larger rod-shaped bacteriamorphs, but were found in the same bacterial films as the rod-shaped forms. Figure 3 is a Scanning Electron photomicrograph of the *nannobacteria* from the GRF.

Another distinctly different form was also observed, that clearly represented a completely different bacteriamorph or nannobacteria. These were filamentous bacteriamorphs, similar to the modern iron-encrusting bacteria, that have been correlated to the occurrence of siderite ($FeCO_3$) in the GRF (Mason, 2008). However, these forms, which have not been positively identified as

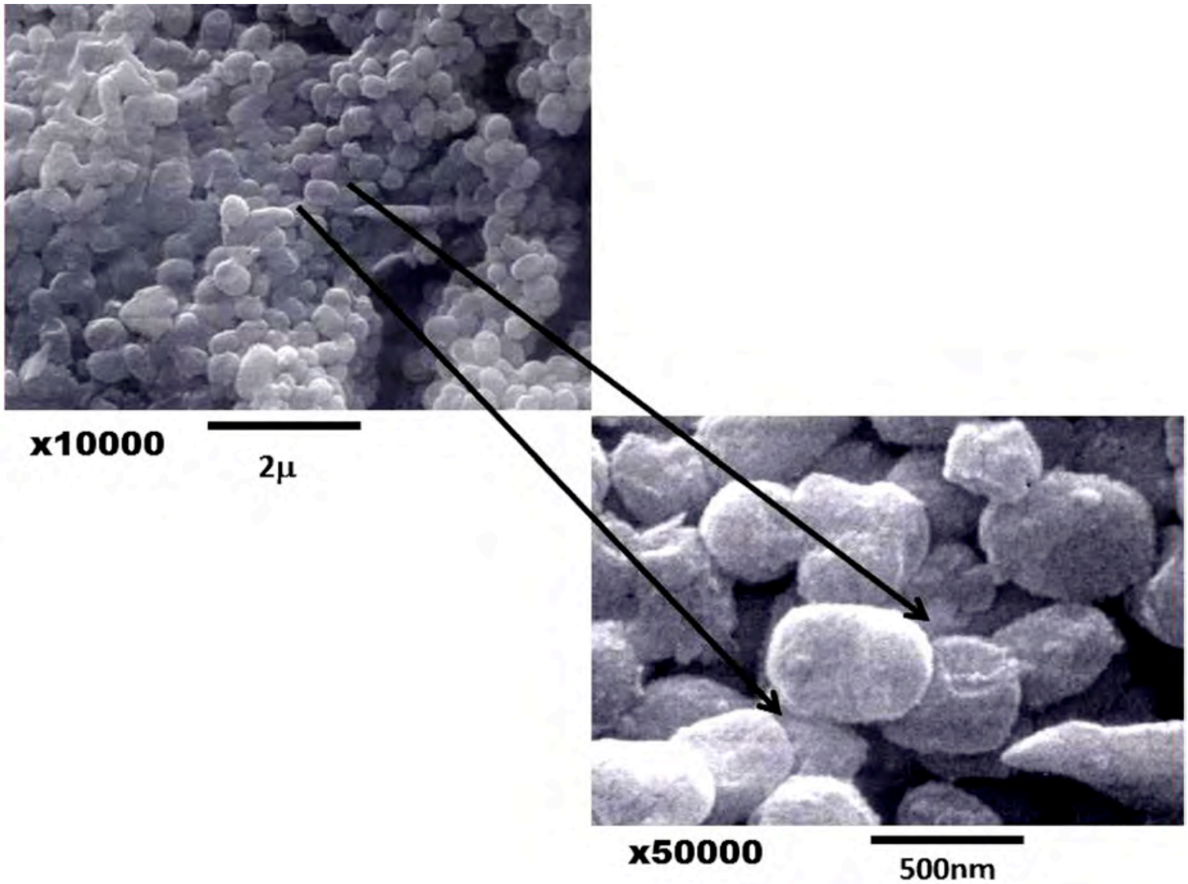

Figure 3. SEM photograph of spherical to oval-shaped fossil nannobacteriamorphs identified from U.S. ERDA Blacks Fork core hole No. 1. Fossilized bacterial film from 1502.7 feet.

bacteriamorphs or nannobacteria, were found on the surface of the much larger filamentous bacteriamorphs (See Figure 4).

Conclusion

The author has examined thousands of mineral samples from the GRF in all its basins, members, and mineral variations. No mineral form has been encountered with morphologies that resemble the bacteriamorphic and nannobacteriamorphic forms displayed in this work. Minerals are crystalline, so by definition they tend to have characteristic shapes with distinctive faces and angles.

These bacteriamorphic forms, by all accounts, are free of angularity and do not have shapes that resemble crystalline morphology. They appear biological in origin, although due to the fossilization process, they are replaced entirely by minerals. However, this is not proof that bacteria-like forms were once living. The absolute proof of this lies with the ability of the biological scientist to test for DNA and observe the internal cellular structure. Obviously, this cannot happen in fossilized material; the evidence simply no longer exists. A recent article in by Young and Martel (2010) attempted to put to rest the supposition that nanobacteria, more specifically modern nanobacteria, could be living entities. The author is

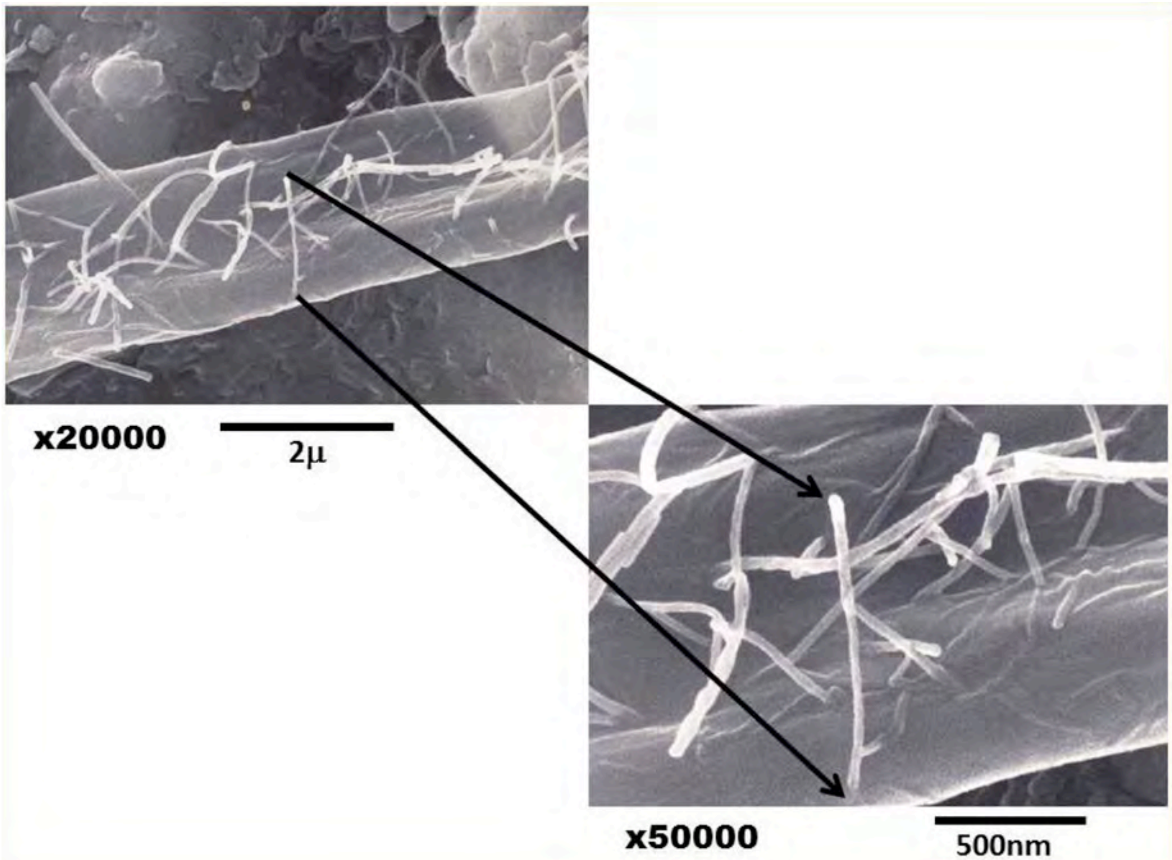

Figure 4. SEM photograph of nano filamentous forms on filamentous bacteriamorphs from U.S. ERDA Blacks Fork core hole No. 1. Fossilized bacterial film from 1651.0 feet.

not sure that this can be applied to these fossil bacteriamorphic forms observed in the GRF.

The Vermin only teaze and pinch
Their Foes superior by an Inch.
So Nat'ralists observe, a Flea
Hath smaller Fleas that on him prey,
And these have smaller Fleas to bite 'em,
And so proceed ad infinitum.
- (Jonathan Swift, 1733)

References Cited

Carroll, A.R., and Bohacs, K. M. (1999) Stratigraphic classification of ancient lakes: Balancing tectonic and climatic controls: Geology, v.27, p. 99 - 102.

Folk, R.L. (1992) Bacteria and nanno-bacteria revealed in hardgrounds, calcite cements, native sulfur, sulfide materials, and travertines (abstract), Geological Society of America Annual Meeting, Program and Abstracts, p 104.

Folk, R.L. (1993) SEM imaging of bacteria and nannobacteria in carbonate sediments and rocks. J. Sediment. Petrol. 63:990–999.

Folk, R. L. (2005) Personal communication.

Mason, G. M. (2005) Fossil Bacteria from the Green River Formation (abstract), Geological Society of America Annual Meeting , Program and Abstracts, p. 205.

Mason, G. M. (2008) Saline Minerals in the Green River Formation, Green River and Washakie Basins, Wyoming, in Proceedings of the 27th Oil Shale Symposium, Colorado School of Mines Press, Golden, Colorado.

McKay, D. S., Gibson, E. K, Thomas-Keprta, K. L., Vali, L. H., Romanek, C. S, Clemett, S. J. Chillier, Z.D.F., Maechling, C. R., and Zare, R. N. (1996) Search for past life on Mars: possible relic biogenic activity in Martian meteorite ALH84001. Science 273:924–926.

Morita, R. Y. (1988) Bioavailability of energy and starvation survival in nature, Can. J. Microbiol. 34:436–441.

Rhodes, M. K., Carroll, A.R., Pietra, J. T., Beard, B.L., Johnson, C. M. (2002) Strontium isotope record of paleohydrology and continental weathering, Eocene, Green River Formation , Wyoming, Geology, V. 30, No.2, p. 167 - 170.

Roehler, H. W. (1993) Eocene climates, depositional environmental geography, Greater Green River Basin, Wyoming, Utah, and Colorado: U.S. Geological Survey Professional Paper, 1506-F, 74 p.

Swift, J. (1733) Swift Poems II. p. 651.

Tissot, P. R. and Murphy, W. I. R. (1960) Physiochemical properties of Green River oil shale: Journal of Chemistry and Engineering Data, v. 5, p. 558-562.

Westall, F. (1999) The nature of fossil bacteria: A guide to the search for extraterrestrial life. Journal of Geophysical Research, Planets, v. 104, p. 16,437-16,451.

Young, J. D. and Martel, J. (2010) The rise and fall of nanobacteria, Scientific American, 302, pp 62 – 59.