Nanobacteria: gold mine or minefield of intellectual enquiry?

Allan Hamilton

Nanobacteria are very small cellular forms which seem to be closely linked with the formation of geological strata. Do they really exist? If so, their impact on our understanding of living systems is potentially huge.

Amongst microbiologists and molecular biologists there is a general consensus that the minimal size for independent viable life forms is likely to be a coccus of approximately 0·2 µm diameter with a volume of 0·06 μm^3. Perhaps not entirely coincidentally, this equates closely with the limits of the optical microscope and with the pore size of so-called sterilization filters, although it is also supported by various theoretical calculations of the minimal space required to accommodate the essential components of cellular life, as we currently understand them.

Nonetheless, from time to time there have appeared reports of various mini-forms such as granules, inclusion bodies, minicells and ultramicrobacteria. With the probable exception of ultramicrobacteria, these various mini-forms have not been extensively characterized, although they generally appear to be associated with conditions of stress and/or infection of higher organisms. The recent emergence of so-called nanobacteria, however, has dramatically re-opened the debate as to the minimal size for viable life forms and brought an urgency to research activity in subject areas as apparently diverse as infectious diseases of humans, microbiology of the deep subsurface and the origins of life.

This is a field as yet more associated with unanswered questions than with firm data and definite conclusions, but it is not a field lacking in strong characters and audacious claims. If even some of the latter can be substantiated then we are indeed sitting on a gold mine of intellectual enquiry, albeit one currently guarded by something of a minefield of circumstantial and uncorroborated findings.

To progress further, we must find the answers to questions that can be set at three different levels of enquiry, of increasing complexity and of widening relevance.

- What are the characteristics used to define nanobacteria?
- What do we know and what remains to be determined to establish their biological nature?
- What roles, if any, do they play in human disease, biomineralization and geological evolution, and the origins of life on this and other planets?

Characteristics and habitats

The major property used in characterizing the various mini-forms, i.e. size, is notoriously difficult to establish with any accuracy and free of artefactual distortion with objects in the 1 µm and less range. It is always possible, therefore, that either a single name can be used to identify two or more phenomena, or that the same basic structure can be given two names depending on the conditions of its discovery. Something of the latter may be the case with the terms ultramicrobacteria and nanobacteria. Ultramicrobacteria have been described as resting cellular life forms, of 0·3 µm diameter, derived from marine bacteria in response to stress, notably starvation. On nutrient supplementation, normal cell size and growth are re-established. More recently Jan Gottschal has proposed that ultramicrobacteria are naturally occurring organisms, again primarily found in the marine environment, which have a marked survival capacity, grow extremely slowly and retain their small size. With the exception of their larger dimension, this description of ultramicrobacteria fits closely with what is envisaged for nanobacteria. At this stage of our understanding, therefore, it might be best to consider the terms as synonymous.

The undoubted champion of nanobacteria (except that he prefers the spelling nannobacteria) is Robert Folk, a distinguished sedimentary geologist from Austin, Texas. Using techniques of acid etching and gentle gold shadowing, Folk has demonstrated the presence of tiny spherical structures (in the size range 0·05–0·2 µm) in an extensive array of geological materials. He has hypothesized that these are microfossils of previously active nanobacteria and that their activities were central to the actual formation of the geological strata in question. Folk has further claimed that evidence for extant nanobacteria is to be found in such diverse environmental samples as tap water and decaying leaves. These data have been published largely in journals and conference proceedings within the geological literature, and Folk has not yet found a particularly receptive audience within the microbiological community.

Potentially a major advance in this last regard came with a publication in *Proc Natl Acad Sci* in 1998 by the Finnish group of Kajander & Ciftcioglu. The authors demonstrated nanobacterial forms in human and cow blood, and in commercial cell culture media. They were able to grow these nanobacteria in normal culture media and to show that the cells laid down deposits of biogenic apatite on their cell envelope. Kajander & Ciftcioglu proposed on the basis of these data that nanobacteria are common organisms within the animal body and may be ultimately responsible for conditions such as tissue calcification and kidney stones. Further, they extracted 16S rRNA from their nanobacteria, and from gene sequence analysis deduced that they belonged within the α-2 subclass of the *Proteobacteria*. Unfortunately, it appears that the Finnish group had experienced a period in the scientific wilderness prior to their paper and that even since that publication their work has met with considerable scepticism in general and, as reported in *Nature* last year, downright antagonism from within the Finnish academic community.

Taking the more charitable view that such criticism and rejection as have been experienced by Folk and the Finnish group owe more to the challenging novelty of their findings than to any inherent fault in their scientific enquiry, we can say that, as a working hypothesis, nanobacteria are defined as extremely small cellular forms, widespread in nature and closely associated with the formation of inorganic precipitates and geological strata.

Moving to the next stage of our analysis, at least three criteria can be used to establish whether nanobacteria are true life forms. Do they demonstrate increase in biomass during incubation in nutrient media? Can evidence be obtained of the presence of nucleic acids? Can cellular structure be demonstrated? Although Folk's geological studies cannot shed any light directly on these questions, the Finnish group has claimed positive answers in each case. Is there any corroboration from other research groups on this key issue?

Another geologist, Philippa Uwins, working at the University of Queensland, Australia, has found filamentous forms associated with various rock samples. These resemble actinomycetes or fungi rather than bacteria, but their very small dimension (0·020–0·128 µm diameter) has caused them to be identified as nanobes. Uwins and her colleagues have been able to demonstrate growth of these nanobes, membrane and wall structures and the likely presence of DNA. These studies have elicited the, by now normal, expressions of scepticism, with the main criticism focussing on the diversity of size and shape which is taken to suggest that the nanobes may be simply fragmentation products of a larger cellular life form.

Nanobacteria in the deep subsurface

My own involvement with and interest in nanobacteria stems directly from the scanning electron microscopy (SEM) picture opposite. This was obtained by my colleagues Carol Devine and Iain Spark as part of a combined microbiological and geological study of reservoir souring and formation damage in the offshore oil industry. It shows fresh sandstone core material taken from a subsea depth of 13,000 feet, after 7 days incubation in nutrient medium at 90 °C. Individual structures are less than 0·1 µm in diameter and they show a striking circular aggregation pattern. These nanobacteria are not evident on core material prior to incubation, nor are they found in the liquid medium at the end of the incubation period; their presence appears to require both nutrient medium and a geological surface. Similar patterns were found after incubation at both 60 and 30 °C, but only after 28 days and 6 months, respectively. At each temperature, increasing the time of incubation gave rise to increasing biomass, as evident in SEM analyses. These nanobacterial forms, uniquely associated with core material from the deep subsurface, therefore show the standard temperature and time dependencies characteristic of cellular biological forms added to nutrient media.

While it must be freely admitted that all the evidence so far available concerning the existence and nature of nanobacteria can best be described by such euphemisms as 'preliminary' or 'indicative', this author would wager that while nanobacteria may be very small, their impact on our future understanding of living systems has the potential to be very large indeed.

The work of the Finnish group places nanobacterial forms as central to a wide range of medical conditions, and notably those associated with the formation of inorganic precipitates. Fortuitously or otherwise, the other groups whose work I have very briefly summarized have seen nanobacteria as agents at least associated with, and possibly even responsible for, many of the geological formations previously assumed to be wholly sterile and to have evolved quite independently of any biological processes. The comparatively recent demonstration that the deep subsurface is, in fact, an extensive and wholly active biosphere in its own right, has clearly shown the error of that assumption. There is even an increasingly

ABOVE:
Circular aggregations of <0·1 µm structures on fresh sandstone core material as described in the text. Bar, 3 µm.
COURTESY CAROL DEVINE AND IAIN SPARK

widespread belief that the surface-associated high-temperature characteristics of the deep subsurface constitute the ideal environment for those initial processes leading to the origins of life itself. All of which takes us neatly to NASA, meteorite ALH84001 and life on Mars!

● Life on Mars?

In 1996 *Science* published a paper suggesting that a Martian meteorite, identified as ALH 84001, carried evidence of previous life on that planet. This striking claim rested on a number of features which together were taken as being compatible with life on Mars: carbonate globules, and magnetite and iron sulphide particles whose chemistries were suggestive of biological processes of formation; the presence of polycyclic aromatic hydrocarbons; and SEM and transmission electron microscope (TEM) images very closely resembling Folk's terrestrial nanobacterial fossils. Perhaps not surprisingly, this publication aroused considerable interest, including a Presidential press conference. Now, however, it has become fashionable in most scientific circles to debunk the original claims as being greatly exaggerated and largely unsustainable. In such an emotive area, where there is a great human desire to find a definite 'yes' or 'no' to a question where all the data available to us must by its nature be indirect and circumstantial, it is important to resist the temptation to take a polarized position. As one of the authors of the original paper has subsequently stated,

> '*It is important to stress that we have not found proof of life on another planet; rather, we observed features that are nearly identical to those we might expect had life once existed on Mars. This may be a fine distinction, but it is an important one*'.

The full nanobacterial story remains to be told, but its resolution and the light it may shed on other great scientific mysteries holds promise of being hugely exciting.

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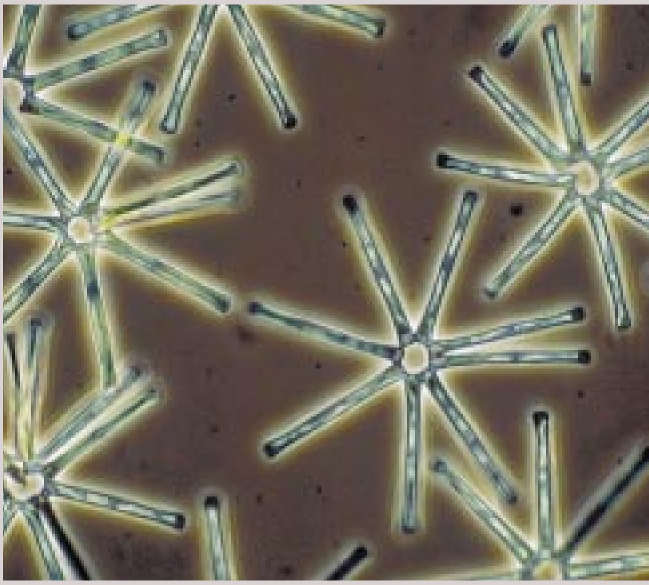
UKFCC logo competition – £250 prize

Micro-organisms, which are exceptionally diverse, are found almost everywhere and affect human society in countless ways. Thus, modern microbiology has a great impact on medicine, agriculture, food science, ecology, genetics, biochemistry and many other fields. The microbial world holds the potential to revolutionize many aspects of human life and culture collections are a key resource that is required to underpin these developments.

It is easy for those not actively involved in microbiology to overlook the importance of culture collections, but without 'biological standards' it would be impossible to perform comparative science, authenticate specimens, guarantee productivity in processes using micro-organisms/cell lines or lodge patents involving micro-organisms. In the UK there is a huge diversity of collections, both large and small, often associated with an individual scientist or research group. Such collections include those in the PHLS, the university sector, industry and the constituent collections of the UK National Culture Collection (UKNCC). The United Kingdom Federation for Culture Collections (UKFCC) aims to serve all those who are involved with or use culture collections.

The UKFCC was established on 10th April 1975 at Imperial College London. Membership of the Federation is open to all those involved with Culture Collections/ Genetic Resource Centres (GRCs) and the users of cultures. Today, its membership includes users of genetic resources from both the academic and commercial sectors, as well as representatives of the constituent collections of the UKNCC. Members receive a twice-yearly newsletter, have reduced course fees on UKFCC training courses and are affiliate members of the World Federation of Culture Collections (WFCC).

In its 25th year the UKFCC has initiated a competition (first prize £250) to design a new logo for the Federation. For further details contact the Secretary of the UKFCC: Dr John G. Day, CEH Windermere, Far Sawrey, Ambleside, Cumbria, LA22 0LP (Tel. 015394 42468; Fax 015394 46914; email jgd@ceh.ac.uk).



Asterionella formosa CCAP 1005/5. COURTESY JOHN DAY