Nannobacteria, Organic Matter, and Precipitation in Hot Springs, Viterbo, Italy: Distinctions and Relevance

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Tiny (50-200 nm) spheroids were first discovered by Folk through SEM work on the hot springs of Viterbo Italy. He termed these small, spherical structures "nannobacteria," and proposed that they may be important agents in precipitation of $CaCO_3$, as needle-like crystals of the mineral aragonite, and as bundles of such needle-like crystals (termed "fuzzy dumbbells"), or as elongated crystals of the mineral calcite [1].

During the past 15 years, nanometer-scale spheroids have been discovered in the geological, medical, and astronomical worlds. There can be no doubt as to their existence, but their significance and origin remain a subject of continuing controversy. Even the spelling ("nanno-"), which has been the standard in biology, geology, and paleontology going back to the 19^{th} century, has been questioned. Whether or not they are truly bacteria or any form of life has been a subject of heated debate. At one end of the spectrum are claims that they are a minute form of microbial life (bacteria or archaea) because they are culturable organisms with distinct cell walls/membranes that stain (+) for DNA. Other workers suggest that they are membrane vesicles blebbing off of the cell walls of "normal" bacteria. Another view is that they represent inchoate mineral nuclei formed either in the presence of bacterial slime (EPS) or in other organic-rich milieus. A key argument of opponents to the concept of very small bacteria is that a minimum volume of 200 nm^3 is thought to be needed to hold all the molecules essential for life functions [2].

These debates have motivated years of research. If nothing else, we have learned that when trying to answer questions at the nanometer scale, careful sample preparation is essential. Unfortunately, during preparation of samples for SEM, spherical nannobacteria-like artifacts can be produced by gold coating for more than 60 seconds. Therefore, we coat our samples for no more than 30 seconds to avoid creation of spherical artifacts [3]. Unfortunately, sub-spherical, nannobacteria-like features can also be produced by acid-etching of purely inorganic minerals [4]. Therefore, we strive to image only freshly broken surfaces and we use acid only t[illegible] samples when we can compare an acid-etched surface to a freshly broken surface. Trials of different dehydration methods (air drying, air drying under vacuum, sequential ethyl alcohol solutions, with HMDS, or critical point drying) also resulted in significant, visible differences in the appearance of the dehydrated biofilm, particularly at the nanometer scale, including tiny, spherical artifacts [5]. Dehydration in acetone produced the best-preserved biofilm structure for our TEM sections. Finally, experiments in which bacterial cultures were lysed and then air-dried resulted in formation of bacterial debris containing nanometer-scale textures [4].

With the remains of lysed bacteria in mind, we established criteria for distinguishing nannobacteria from nanometer-scale textures in fine-scale organic debris. In SEM, nannobacteria appear spherical and may be associated with mucilage. Nannobacteria tend to cluster, are consistent in size, are truly spherical to ovoid, have a consistent, if not smooth, surface in SEM and in TEM show an intact cell wall (100 nm in diameter) (Fig. 1 A&B). In TEM nannobacteria with cell walls and ribosomes are found in the range of 150-400 nm in diameter. Cells with cell walls/membranes and electron clear interiors are abundant in the range of 50-100 nm in diameter. In contrast, nanometer-scale textures associated with organic debris vary in size locally, are irregular in shape and surface texture in SEM, and show random distribution of amorphous organic matter in TEM.

At the nanometer scale, both nannobacteria and the very fine-scale organic debris associated with them appear to play a role in initiation of mineral precipitates in hot springs and other environments. Hot springs near Viterbo, Italy (40 km north of Rome) are literally filled with mineral precipitates, mainly aragonite and calcite (polymorphs of $CaCO_3$). The mineral aragonite precipitates as elongate needle-like crystals in conical splays (botryoids), spheres, or double conical splays (fuzzy dumbbells) (Fig. 2 A-C). In some cases crystallization appears to initiate on organic matter. For example, a sample embedded in paraffin, sectioned, stained with safranin and fast green, and examined under a brightfield microscope shows that aragonite precipitation initiated on organic

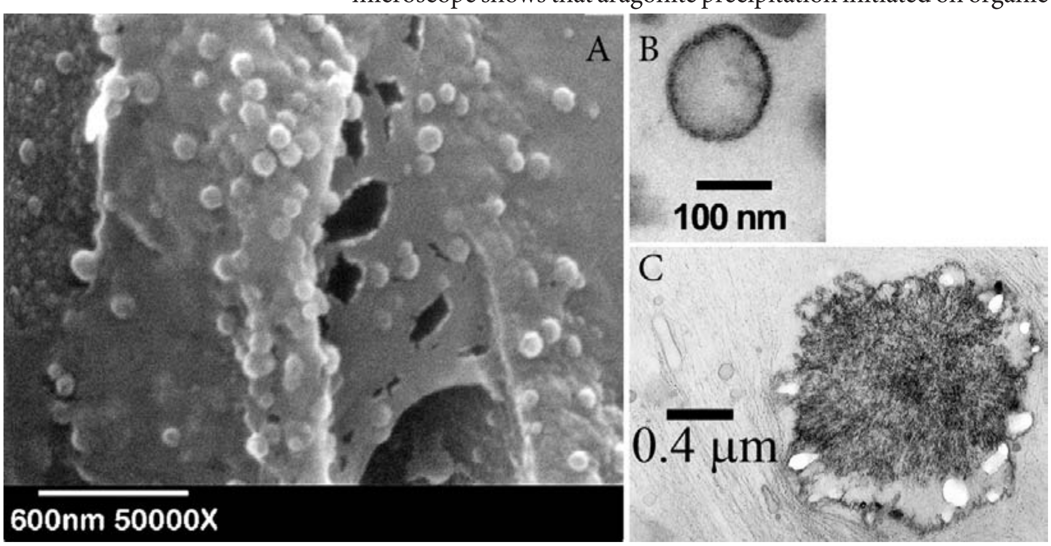

Figure 1. A. SEM photomicrograph of nannobacteria in mucilage from Viterbo hot spring. Nannobacteria and organic textures in background; B. TEM photomicrograph of a single nannobacteria cell showing cell wall; C. TEM photomicrograph of organic matter (dark) and open spaces left after aragonite crystals dissolve (white).

https://doi.org/10.1017/S155192950006243X Published online by Cambridge University Press

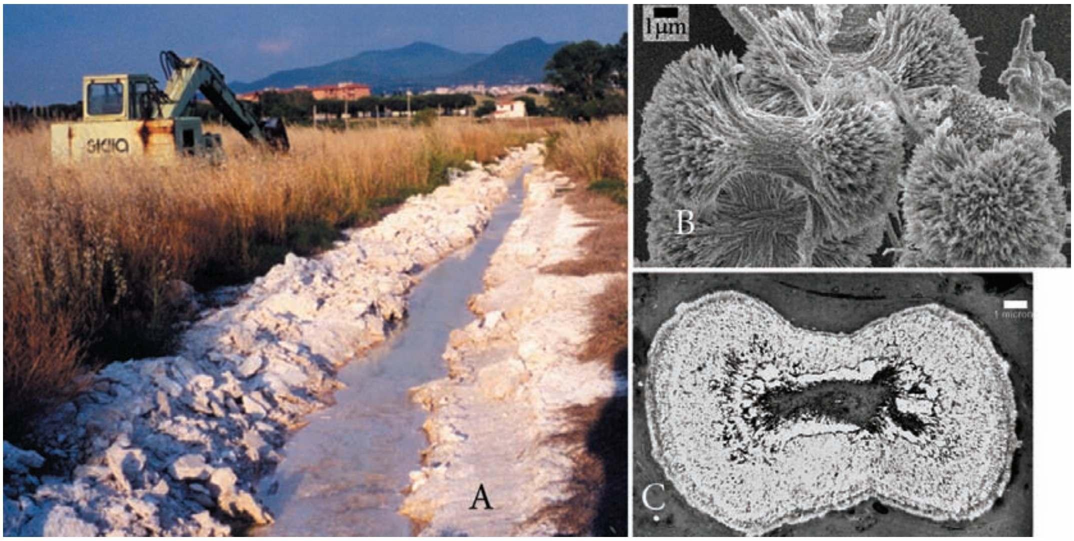

Figure 2 A, A view of travertine deposits (white) associated with hot springs near Viterbo, Italy approximately 40 km north of Rome. The hot springs are related to volcanoes (visible in the distance). The water is very hot (62 C) and saturated with respect to calcium carbonate, hence the abundant precipitation of carbonate minerals such as aragonite. B. SEM photomicrograph showing aragonite in the form of fuzzy dumbbell. C. TEM photomicrograph of fuzzy dumbbell showing amorphous organic matter stained by osmium.

matter. TEM samples fixed with osmium, embedded in resin, sectioned, stained with uranyl acetate and lead citrate, reveal organic matter in the interior of fuzzy dumbbells and aragonite spheres. Under very high magnification SEM (100,000X), the centers of aragonite precipitates contain minute (50-100 nm), spherical structures that we term "nannobacteria". In addition to spherical nannobacteira, organic debris dominates the interior of fuzzy dumbbells and botryoids. The distal parts of the aragonite botryoids are composed of smooth needles, which is exactly what we would expect in inorganically precipitated crystals.

On the other side of the world, in a completely different rock and in a very different environment, a remarkably similar distribution of nanometer-scale textures is found in the core of botryoidal hematite (iron oxide) from well-lithified red layers in sandstone units from Mississippi. These observations lead us to conclude that nannobacteria and the organic mucilage associated with them are the point of initiation of precipitation in both aragonite botryoids from Italian hot springs and hematite botryoids from Mississippi sandstones; two very different minerals in two very different environments, but with similar organic origins.

Identifying nannobacteria and determinining the relative significance of nannobacteria and their associated organic matter to precipitation remains the focus of further research. From a geological perspective, this work may be significant for understanding the origin of some of the mineral components that cement sandstone and limestone, thus affecting the quality of petroleum reservoirs and aquifers. The most heated debates over nannobacteria are documented in the medical literature [e.g. 6, 7]. Nannobacteria have been postulated to cause human arterial disease [7] (Fig. 4 A and B). Perhaps less urgent, but equally exciting, is the postulation of the tiny, humble nannobacteria as being the first visible evidence of life on Mars from the Martian meteorite ALH8400 [8]. It is important to determine what these nannosperoids really are. Are they some form of life, products of living organisms, or inorganic? Are they the cause of much human disease, harbringers of extraterrestrial life or mere curiosities? ■

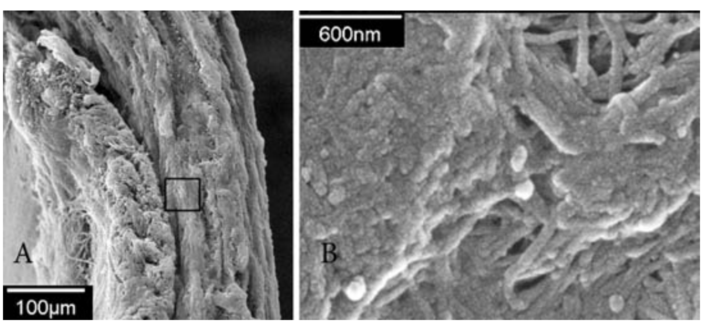

Figure 3. A. Section through wall of human aorta.
B. Close up of area outlined by box in figure A. Note small, ovoid structures strikingly similar in shape and size to nannobacteria grown in culture by Mayo Clinic researchers.

Acknowledgements

This work was supported in part by NSF SUGER grant EAR-9803031 and would have been impossible without the able staff of the Electron Microscopy Center at Mississippi State University.

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https://doi.org/10.1017/S155192950006243X Published online by Cambridge University Press