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REVIEW

Ultramicrobacteria: Formation of the Concept and Contribution of Ultramicrobacteria to Biology

V. I. Duda[a, b, 1], N. E. Suzina[a], V. N. Polivtseva[a, b], and A. M. Boronin[a, b]

[a] *Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences, pr. Nauki 5, Pushchino, Moscow oblast, 142290 Russia*

[b] *Pushchino State Natural Science Institute, pr. Nauki 3, Pushchino, Moscow oblast, 142290 Russia*

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Abstract—Ultramicrobacteria (UMB) are species of the domain Bacteria characterized by very small sizes of proliferating cells (less than 0.1 μm^3 in volume) and small genomes (3.2 to 0.58 Mb). Some authors use the term nanobacteria as a synonym of UMB. Several tens of UMB species have been isolated from various natural habitats: sea water, soil, silt, Greenland ice sheet, permafrost soils, and intestines of humans and insects. Under laboratory conditions, they are cultivated on different nutrient media. In the second prokaryotic domain, the *Archaea,* ultrasmall forms (ultramicroarchaea) have also been described, including nanoarchaea (members of the genus *Nanoarchaeum*) with a cell volume of less than 0.1 μm^3. The term nanobacteria is used in the literature also to denote ultrasmall bacterium-like particles occurring in rocks, sands, soils, deep subsurface layers, meteorites, and clinical samples. The systematic position and the capacity for self-reproduction of these particles are still unclear. The cultured UMB forms are characterized by highly diverse morphology, ultrastructural organization, physiology, biochemistry, and ecology. UMB form three groups according to the type of cell wall structure and the reaction to Gram staining: (1) gram-negative, (2) gram-positive, and (3) cell wall-lacking. Their cells divide by constriction, septation, or budding. The unique processes performed by UMB are dehalorespiration and obligate or facultative epibiotic parasitism. The UMB that synthesize organic compounds in ocean waters with the involvement of proteorhodopsin play a great role in the biosphere. UMB have been found in seven large phylogenetic groups of prokaryotes, where their closest relatives are organisms with larger cells typical of bacteria, which is evidence of the polyphyletic origin of the currently known UMB species and the reductive mode of their evolution.

Keywords: ultramicrobacteria, nanobacteria, ultramicroarchaea/nanoarchaea, ultramicrocells, nanocells, microbial minimization, intermicrobial parasitism, reductive evolution in bacteria

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INTRODUCTION

Bacterial species exhibit great variations in cell size, the cell volume ranging from 0.02 to 180000000 μm^3. The part of this spectrum encompassing ultrasmall values is occupied by ultramicrobacteria (UMB). While the smallest spheroid eukaryotic algae are 0.6–2.0 μm in diameter [1, 2], the minimum diameter of coccoid UMB cells is only 0.15–0.2 μm, i.e., 150–200 nm (see review by Cavicchioli and Ostrowski [3]).

The understanding of the UMB nature, evolution, and role in the biosphere is closely associated with the solution of such problems of microbiology and general biology as the essence and mechanisms of microbial cell minimization, the minimum permissible sizes of autonomously reproducing living cells, the origin of primordial living organisms, the possibility of using UMB as nanobacteria in nanobiotechnology, and other problems.

[1] Corresponding author; e-mail: duda@ibpm.pushchino.ru

The use of UMB (mycoplasmas) as model organisms was of crucial significance in the epoch-making works of J. Craig Venter and his colleagues [4, 5] on experimental production of a novel mycoplasma species and creation of a novel living being with cells containing chemically synthesized chromosomal DNA.

Based on direct microscopic observations, microbiologists long ago arrived at a conclusion that natural habitats are dominated by very small coccoid microbial cells with a diameter of 0.3–0.5 μm [6–11]. However, only single bacterial species with ultrasmall cells, strictly satisfying the criteria for UMB, have so far been isolated as pure cultures. This considerably restricts the possibility of estimating of the role of UMB in biosphere processes. The isolation failures are supposedly caused by the discrepancy between the natural conditions of UMB growth and development, and the laboratory nutrient media and cultivation conditions. The terminology used for characterizing UMB properties is highly variegated and contradictory. Hence, the goal of this review is to analyze the

state-of-the-art of the UMB research, critically appraise the data obtained, and highlight the prospects of investigations in this field of microbiology.

Terminology

Ultramicrobacteria. The term ultramicrobacteria (UMB) was first used by Torrella and Morita to describe extremely small bacteria of less than 0.3 μm in diameter [6]. These bacteria were isolated from sea water and formed ultramicrocolonies on agar plates. The isolates grew very slowly, even at high concentrations of nutrients in the agarized media, and retained their small cell size. The species affiliation of these microorganisms remained unclarified. MacDonell and Hood [11] expanded the description of the UMB group by adding the bacteria that they obtained by filtration of river water through a membrane filter. These bacteria formed normal-sized colonies on agarized media. Schut et al. [12] modified UMB definition by including a cell volume index. According to these authors, this index must be less than 0.1 μm^3 and should not vary with growth conditions. In addition, the authors introduced one more criterion for UMB: small size of the genome. The list of indices for classification of organisms as UMB was also presented in Velimirov's work [8].

Thus, the modern concept of UMB is based on the taxonomic (species) principle of diagnostics with taking into account the affiliation with UMB by the following mandatory criteria: (1) ultrasmall sizes (the volume of less than 0.1 μm^3 for most cells in the populations); (2) maintenance of ultrasmall cell size regardless of the growth conditions and culture development stage; and (3) small genome size (~3.2 to ~0.58 Mb). Later on, researchers adhered to these criteria when describing UMB in their publications. Unfortunately, the term UMB has also been used in some publications to denote ultrasmall cells of indigenous microorganisms observed in situ in natural habitats or obtained from natural samples by methods of differential centrifugation or filtration through 0.4- to 0.1-μm pore-size filters. This results in confusion of the concepts of ultrasmall cells and UMB as a microbial type. One should bear in mind that the fractions of ultrasmall cells of indigenous microorganisms may contain nonreproducing old resting cells, cyst-like forms, nucleus-free minicells, pathological degenerative cells, wall-less cells, cytoplasmic fragments, periplasmic vesicles surrounded with the outer membrane, and other bacteriomorphic particles [7, 13–15]. Only some of these cells may represent actual UMB species. The species affiliation of uncultivated UMB and of UMB observed in situ in natural habitats can be established by the methods of molecular systematics and microscopic analyses.

The term "ultrasmall bacteria" or "ultrasmall cells" is used in the literature when the authors cannot present accurate parameters of dimensions or when the cell size value typical of UMB is slightly exceeded. It should be noted that the upper limit of the volume (0.1 μm^3) has not yet been substantiated in biological terms and is based on the precedent of description of the first sufficiently characterized UMB species, *Sphingopyxis alaskensis* (formerly *Sphingomonas alaskensis*) [12]. There is no distinct dimensional hiatus between UMB and the species with somewhat larger cells. However, it currently seems reasonable to adhere to the above criterion when describing novel UMB species, although this attitude may be modified after description of a larger number of novel UMB species and establishment of their biological peculiarities. It should also be taken into account that the studied UMB species differ in cell polymorphism. Hence, it is reasonable to designate the species with homogeneously small size of cells in the population as obligate UMB, while the species whose growing cultures contain a small proportion of cells with a volume larger than 0.1 μm^3 should be referred to as facultative UMB. The examples of obligate UMB are *"Pelagibacter ubique"*, *S. alaskensis*, and the representatives of the class *Actinobacteria* isolated by Hahn et al. [40].

Ultramicroarchaea (nanoarchaea) and ultramicroprokaryotes. The domain *Archaea* also includes species with ultrasmall cells (nanocells) [16, 17], and they obviously are to be denoted by a term close in its meaning to the term UMB. In our opinion, it could be ultramicroarchaea (UMA), while ultramicroprokaryotes (UMP) seems to be a reasonable term for combining these two groups of microorganisms. For the first recently described representative of nanoarchaea, the name *Nanoarchaeum equitans* has been proposed. Distinctive features of this organism are as follows: spherical cells of 0.4 μm in diameter, extremely small genome size (0.58 Mb), and obligate exocellular parasitism [16]. Other UMA are also known, but they have not yet been described at the species level [17].

Nanobacteria. The term nanobacteria (NB) has different interpretations.

1. UMB synonym. Morita [18] was the first to use the term NB as a synonym of UMB. Some other authors also use the term NB as a synonym of UMB [3, 8, 10].

2. According to Folk [19], NB are indigenous forms of coccoid bacterial cells occurring in geological rocks (sands, grounds, silts, sedimentary rocks, etc.) and characterized by ultrasmall sizes (0.3–0.1 μm and less in diameter). In geological literature, the term NB is also used for description of microfossils, the origin and nature of which are currently interpreted in a speculative manner.

3. According to Kajander and Ciftcioglu [20], NB are representatives of human-pathogenic bacteria of the genus *"Nanobacterium"*, whose cells measure 0.3–0.05 μm in diameter. The existence and biological nature of these "bacteria" have not been proved experimentally; therefore, the point of view of these authors

is currently considered merely speculative and is not accepted.

Ultramicrocells. The term ultramicrocells (UMC) has the following synonyms: nanocells, dwarf cells, midget cells, nanosized cells, and is used for ultrasmall coccoid cells 0.4–0.15 μm in diameter occurring in (a) natural habitats; (b) pure UMB cultures (permanently); and (c) old or starved cultures of ordinary bacteria. The term UMC is often used in the literature for designating bacterial cells that have passed through filters with 0.4-, 0.3-, and 0.2-μm pores. Sometimes these cells are incorrectly termed ultramicrobacteria (UMB); in this case, differences are ignored between the cellular and species aspects of these concepts.

Thus, there are two types of ultrasmall cells: the cells of UMB species, and small cells that pass through ultrafilters or are observed using various kinds of microscopy (UMC).

Since the existence of true UMB in natural habitats and microbial communities has been proved, the central task of microbial ecology in this respect is to determine the UMB to UMC ratio and their physiological states in particular biotopes.

Marine ecologists classify plankton fractions by size in the range of 0.02 to 200 μm [21] using the terms femto-, pico-, nano-, and microplankton to distinguish the classes of objects with a tenfold difference in size. Among the classes of this classification, UMB are most close to femtoplankton [21]. However, microbiologists use the prefix "nano" in relation to objects measuring tenths and hundredths of a micrometer (mainly, 0.4 to 0.1 μm), while in marine ecology the dimensions of nanoplankton objects are in the range of 0.2–2 μm.

UMC in Natural Habitats

UMC detection in and UMB isolation from sea waters and continental water bodies. Microscopic analyses show the presence of ultrasmall cells (0.02–0.12 μm^3) in samples of natural substrates [14]. However, after plating these samples onto agarized media, the grown colonies contain cells of 0.34–6.4 μm^3 in volume. Obviously, many of the large cells in this case originate from the former UMC. Hence it follows that UMC are easier to isolate than UMB. This phenomenon may be caused either by the low quantity of UMB or by the necessity of using special media and conditions for UMB cultivation. Thus, it is known that UMB prevail under oligotrophic conditions typical of the open ocean, where the concentration of microorganisms is ~10^5–10^6 cells per ml [9, 12]. However, the ratio of UMB cells to the total number of ultrasmall cells in natural samples is still an open question.

Haller et al. [22] investigated the bacteria obtained by filtration of the Mediterranean Sea water through an ultrafilter with 0.2-μm pores and arrived at a conclusion about the dominance of bacteria that are potentially common-sized but are represented by starvation forms. The filtered microorganisms were assigned to the following phylogenetic groups: *Alphaproteobacteria, Gammaproteobacteria*, and *Cytophaga–Flavobacterium–Bacteroidetes*.

To date, only two new UMB species have been obtained as cultivated isolates from marine habitats: *Sphingopyxis alaskensis* (formerly *Sphingomonas alaskensis*) [12] and *"Candidatus* Pelagibacter ubique" [23–25]. The description of these UMB, widespread in marine waters, represents a notable discovery in microbiology, which has cardinally changed the concepts of UMB biology and role in the biosphere. The research into the biology of *Sphingopyxis alaskensis* played a key role in the formation of the UMB concept (see reviews [3, 26]).

UMC were also obtained from deep groundwater embedded in sedimentary and granite rocks by filtration through ultrafilters with 0.1- or 0.2-μm pores [27]. Phylogenetic analysis of these microorganisms showed that the bacteria that passed through filters with 0.2-μm pores were representatives of *Betaproteobacteria*, while those that passed through 0.2-μm pore-size filters and were then captured by 0.1-μm filters were close to bacteria of the candidate divisions OD1 and OP11, representing novel phylogenetic lineages. However, the possible presence of UMB among these filterable microorganisms was not studied.

Silbaq [28] obtained a fraction of ultrasmall cells by filtration of drinking water through filters with 0.2- and 0.1-μm pores and arrived at a conclusion that the resultant bacteria were not UMB but UMC, since they all yielded colonies with large cells when plated onto rich media.

UMC detection in and UMB isolation from glaciers and permafrost soils. The study of UMC from glaciers and permafrost soils is important in the context of the possibility of UMB survival over long geological periods. Miteva and Brenchley [29] investigated microorganisms embedded in a 120 000-year-old Greenland glacier ice core. Ice samples taken from a depth of 3043 m were shown to contain a large microbial population dominated by UMC less than 0.1 μm^3 in volume. These UMC were obtained by water filtration through filters with 0.4-, 0.2-, and 0.1-μm pores and could be either truly small organisms or smallest starvation forms of common-sized bacteria. Later on, the authors succeeded in isolating pure cultures of two novel species of gram-negative UMB, named *Chryseobacterium greenlandense* [30] and *Herminiimonas glaciei* [31]. In addition, Loveland-Curtze et al. [31] isolated from ice samples of the Greenland glacier a number of bacterial strains of the genus *Microbacterium* with cell sizes close to those of UMB. Miteva and Brenchley [29] assume that, owing to their ultrasmall sizes, UMB are able to develop in the tiny ice cracks inaccessible to bacteria with usual cell sizes.

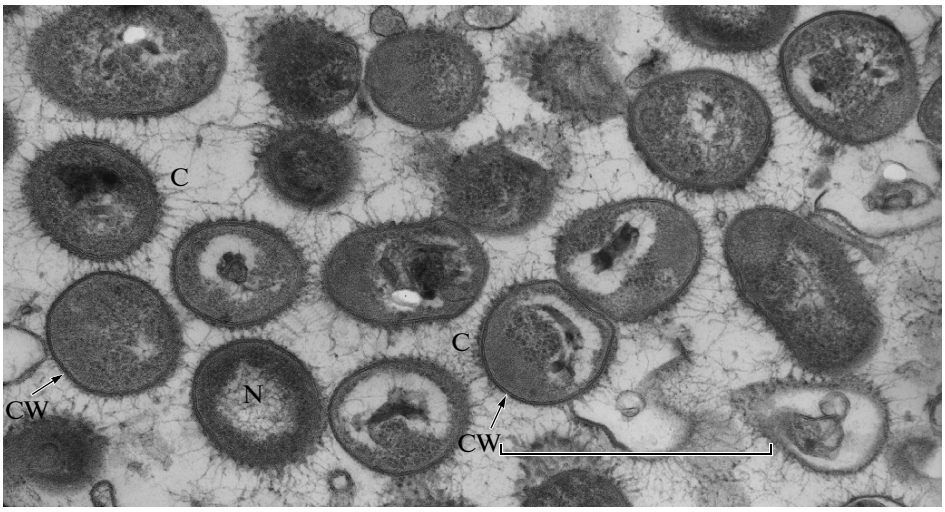

Fig. 1. Thin sections of the cells of *Microbacterium* sp., strain NF7. CW, murein cell wall; C, fibrillar capsule (C); N, nucleoid. Bar, 1 μm.

Dmitriev et al. [13–15] used electron microscopy to study the indigenous microorganisms from 1- to 3-million-year-old permafrost soils of Eastern Siberia. The experiments were performed with samples from permafrost layers that had permanently stayed at a temperature of –10 to –12°C. Microorganisms were extracted from the samples by an original method of low-temperature (at –12°C) cell fractionation by differential centrifugation. The advantage of this method is that it yields native cells of unchanged size and ultrastructure. It has been shown that, among these cells, UMC (spherical or oval forms of about 0.3–0.5 μm in diameter) make up 40–46% of the populations.One of the UMB isolates (Fig. 1) was assigned, based on phenotypic characteristics and 16S rRNA gene sequence analysis, to a new species of the genus *Microbacterium* (Duda et al., manuscript in preparation). These data demonstrate high resistance of UMB to unfavorable environmental factors and their long-term survival under these environmental conditions.

UMC detection in and UMB isolation from soils and sedimentary rocks. Direct examination of natural substrate samples and/or UMC fractions obtained from these samples under light and electron microscopes makes it possible to determine the sizes of indigenous ultrasmall cells. Electron microscopy is of particular value therein, because it ensures not only accurate size measurement but also determination of the physiological status of cells with the use of a complex of tests and criteria developed by cytologists.

The cells with volumes corresponding to those of UMB are persistently observed in soil samples. Data especially important in this respect were obtained in the pioneering works of Bae, Cota-Robles, and Casida [7], as well as Balkwill and Casida [32], who used not only the high-resolution methods of electron microscopy, but also specially developed techniques for separation and concentration of the cells of indigenous microorganisms. The detailed studies of ultrasmall soil forms conducted by these authors revealed two predominant fractions: cells of 0.16–0.3 μm in diameter (40%) and the cells of 0.16–0.5 μm in diameter (88% in some samples). Taking into account that these cells were shaped like coccobacilli, their volume must be less than 0.1 μm^3.

In studies that used analogous methodical approaches, Dmitriev et al. [13–15] obtained similar results and showed that the share of small cells (nanoforms of <0.1 μm^3) was more than 30%. Cells with a diameter of ~0.3 μm and less and a volume of ~0.014 μm^3 and less were assigned by the authors to nanoforms. Some of these forms may be certainly assigned to the category of UMB based on such characters as multiple (repeated) cell division and the peculiarities of ultrastructural organization. Thin sections of soil micromonoliths contained bacterial aggregates: spherical clusters of repeatedly dividing ultrasmall cells, surrounded with a big common capsule (Fig. 2). These coccoid cells were 180–220 nm in diameter. There was no direct contact between these cells and bacteria of other species; therefore, this cluster-forming bacterium can be defined as free-living. This bacterium was not obtained as a pure culture; however, the isolates of two other bacterial species were obtained from the soil samples under study and characterized as UMB [44, 45]. These isolates proved to be members of the genera *Kaistia* and *Chryseobacterium*.

Pure cultures of soil UMB were also obtained by other researchers. Janssen et al. [33] reported three anaerobic heterotrophic UMB isolates from rice soils and assigned them to the order *Verrucomicrobiales*. The cells of these UMB retained their ultrasmall size (0.03–0.04 μm^3) even when grown on rich nutrient

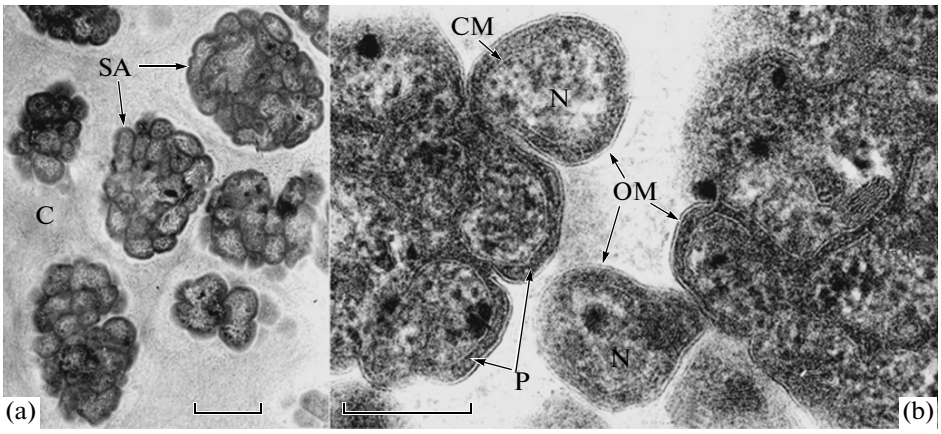

Fig. 2. Electron micrographs of a thin section of a soil micromonolith: (a) spherical aggregates (SA) formed by UMC aggregates surrounded with a capsule (C) and (b) UMC, budding and separating from the aggregates, shown at a higher magnification. Cell ultrastructures: OM, outer membrane; P, periplasm; N, nucleoid; CM, cytoplasmic membrane. Bars, 0.5 μm (a) and 0.2 μm (b).

media. It should also be emphasized that the isolates were obtained from highly diluted soil suspensions, which demonstrates the abundance of these UMB in the soils studied. Iizuka et al. [34] isolated a number of heterotrophic aerobic bacteria with a cell volume of 0.07 to 0.22 μm^3 from polluted soils in Japan. The strain isolated by Sahin et al. [35] from a cell population obtained from urban soil by the membrane filtration method was identified as a novel species, *Oxalicibacterium solurbis*.

The methods of molecular biology are of great importance for estimation of the taxonomic diversity of soil UMB. Rutz and Kieft [36] isolated DNA from the dwarf forms of bacteria and archaea inhabiting semiarid soils and obtained by filtration through a filter with 0.45-μm pores. The results of cloning and sequencing of 16S rRNA genes showed that the dwarf archaeal forms were members of the *Crenarchaeota* phylum, while the dwarf bacteria represented four lineages: *Proteobacteria, Firmicutes, Actinobacteria,* and TM-7 division. Lysak et al. [37] investigated the distribution of cells that could pass through filters with 0.3-μm pores in some soils using 16S rRNA-specific fluorescently labeled probes. They revealed high abundance of members of such prokaryotic groups as *Archaea, Actinobacteria, Cytophaga*, and *Proteobacteria*. Panikov [10], based on the analysis of his own experimental data and data of other researchers, concludes that functions of nanosized bacteria "are diverse and may include qualitatively unique processes and reactions that have their own value, even without huge numbers."

Clinical UMB and NB species. Clinical UMB isolates belong to various taxonomic groups. Most of them are intracellular animal parasites and pathogens of the genera *Brucella, Mycoplasma, Rickettsia*, and *Chlamydia*. In 1998, the existence of peculiar ultrasmall "bacteria" pathogenic to humans was reported, which were designated by the authors as nanobacteria [20]. These organisms were assigned to a new genus and species *"Nanobacterium sanguineum"*. However, no reliable evidence of the cellular nature of these NB was obtained. The data on the 16S rDNA nucleotide sequence of *"Nanobacterium sanguineum"* were later recognized as erroneous (obtained as a result of contamination with usual bacteria) [38, 39]. Experimental studies showed that the supposed nanobacteria were organo-mineral particles. It should also be noted that the above genus and species names have not been validated (they have not been published in the IJSEM Validation Lists).

New UMB species described recently. Over the last decade, new UMB species were discovered and described that have substantially changed the knowledge of the diversity of this group of organisms and their role in natural ecosystems.

1. Giovannoni and coworkers [23, 24] described a new UMB species which they named *"Pelagibacter ubique"*. This free-living gram-negative marine oligotrophic bacterium has vibrioid cells of very small size, ~0.01 μm^3 in volume. It is also has a small genome of 1.3 Mb. The data obtained from the analysis of the complete genome sequence [25] demonstrate the absence of transposons. Extrachromosomal elements are absent as well, and there are almost no non-coding sequences. Thus, the genome of *"Pelagibacter ubique"* is characterized by utmost rationality. The utmost compactness and simplification (streamlining) of the genome is in agreement with the ultrasmall cell size. A physiological peculiarity of this UMB is that it uses proteorhodopsin to utilize the energy of light in the course of unusual photosynthesis and assimilates the carbon of organic compounds dissolved in seawater. This property allows it to produce large biomass: the population of this UMB reaches up to 25% of the total microbial population in ocean waters [23–25].

2. Of fundamental significance was the isolation by Hahn et al. [40] of the four strains of aquatic actino-

bacteria and their detailed description as UMB These organisms are the first well-characterized UMB with the gram-positive cell wall structure. Its selenoid cells are steadily ultrasmall (<0.1 μm^3). The isolates were assigned to the family *Microbacteriaceae* within the class *Actinobacteria*. The physiological peculiarity of the isolates is their weak slow growth and formation of very small colonies on agarized media.

3. The anaerobic UMB strain DF-1, isolated from estuarine sediments in Charleston Harbor, is capable of dehalorespiration on polychlorinated biphenyls (PHB) [41]. Another physiological peculiarity of the isolate is that it requires the presence of *Desulfovibrio* for growth in a coculture or cell extract for growth on hydrogen and PHB. Strain DF-1 is a close relative of bacteria of the genus *Dehalococcoides*. These data are important in the context of the possibility of developing a method for detoxification and elimination of pollutants such as PHB.

4. The recently isolated and described ultramicrobacterium *"Elusimicrobium minutum"* is the first cultivated member of the termite group 1 bacteria [42]. The distinctive features of this UMB are as follows: gram-negative cell wall structure, growth as thin rods 0.3–2.5 μm long and 0.17–0.3 μm thick, and obligate anaerobiosis. Recently, the complete genome sequence of *"Elusimicrobium minutum"* (1.64 Mb) was determined [43]. This UMB is considered to be a member of the novel bacterial phylum "*Elusimicrobia*".

5. The group of strains NF1, NF3, NF4, and NF5 was isolated from soils and lake sediment and assigned to UMB [44, 45]. These are gram-negative nonmotile coccobacilli belonging to two genera, *Kaistia* and *Chryseobacterium*. Their feature that is of particular interest is the facultative capacity to parasitize some bacterial species. Strains NF1 and NF3 are phylogenetically close to the species *K. adipata*, while NF4 and NF5 are close to the soil bacterium *Chryseobacterium solincola*.

The currently available data show great diversity of microorganisms of the UMB group: it includes both free-living heterotrophs and parasitic forms, bacteria with gram-positive and gram-negative types of cell wall structure, and wall-less species. The group includes members of seven deep phylogenetic lineages of bacteria. Thus, neither gram-negative nor gram-positive or archaeal types of structural and molecular organization of the cell are prohibitive characters for UMB or UMA emergence in the course of the evolution.

Ecology and Physiological–Biochemical Peculiarities of Cultivated UMB

The currently known UMB are members of different physiological groups of bacteria: they include aerobic and anaerobic species, free-living oligotrophs as well as symbiotic and parasitic forms, bacteria with cell walls of gram-positive and gram-negative structure and mycoplasmic (wall-less) forms. Oligotrophic UMB include *Sphingopyxis alaskensis*, the properties of which are described in detail in the review [26]. Another UMB with pronounced oligotrophic metabolism is the marine bacterium *"Pelagibacter ubique"* [23].

Intermicrobial parasitism. Ultrasmall cells impart certain ecological advantages to the species possessing these forms: such microorganisms spread more easily in natural habitats as they occupy econiches inaccessible to other species [46]. Size reduction causes an abrupt increase in the ratio of the cell surface area to cytoplasmic volume, and this promotes rationalization of the nutrient transport and efficient assimilation of organic carbon from diffused state, which is highly important for oligotrophic microorganisms inhabiting environments with low concentrations of nutrients. Small cells are particularly important for parasites that need direct physical cell–cell contacts (a kind of epibiosis). It is obvious that the host cell must be larger to provide the development of numerous adsorbed parasitic forms. Indeed, ultrasmall cells with much lesser sizes than the cell size of their prey are characteristic of most of the known parasitic prokaryotes. Ultramicroprokaryotes with a cell volume of <0.1 μm^3, both obligate parasites of microorganisms and facultative parasites, have been found in different phylogenetic and taxonomic groups of bacteria. Some of these species are characterized by the epibiotic lifestyle [44, 45, 47–50] (table). Alphaproteobacteria of the genus *Kaistia* [44] and members of the phylum *Bacteroidetes* (*Chryseobacterium*) have been described as UMB that are epibiotic parasites [45]. It has been shown that strains NF1 and NF3 can parasitize living cells of some species of gram-negative and gram-positive heterotrophic bacteria, as well as cyanobacteria (Figs. 3–6), while strains NF4 and NF5 can parasitize the gram-positive bacterium *Bacillus subtilis*. Only one of the known bacterial species (*Vampirovibrio chlorellavorus*) is an obligate parasite (of the alga *Chlorella*) [48]. The cell size of this microorganism is close to that typical of the known UMB forms. When parasitizing their prey, the above UMB establish tight cell–cell contacts with it. In strains NF1 and NF3, cone-shaped prosthecae on the cells serve as adhesive structures. A unique feature of strains NF1, NF3, NF4, and NF5 is the presence of polysaccharide trapping networks on their cells, with their threads having adhesive granules (Figs. 5–7). The parasites trap prey cells with this network and pull up to them, taking advantage of the Brownian movement forces. This mechanism is crucially important for these parasites because these organisms are nonmotile and, hence, incapable of chemotaxis.

Ultrasmall prokaryotes that are epibiotic parasites of microorganisms

Organism, type of parasitism	Phylogenetic group	Genome size (Mb)	Cell morphology and sizes (μm)	Presence of flagella and chemotaxis
1. *Bdellovibrio* spp., obligate parasite	δ-*Proteobacteria*[1]	3.78[5]	Vibrios, 0.2–0/50/5–2.5 μm ($V \sim 0.13$ μm^3)	+
2. *Micavibrio admirandus,* obligate parasite	α-*Proteobacteria*[1]	n.d.	Vibrios, 0.3 × 0.8 μm ($V = 0.05$ μm^3)	+
3. *Vampirovibrio chlorellavorus*, obligate parasite[6]	n.d.	n.d.	Vibrios and cocci, diameter of 0.3–0.6 μm	n.d.
4. *Kaistia adipata*, str. NF1, NF3, facultative parasites	α-*Proteobacteria*[2]	~2.4[3]	Coccoids, 0.2–0.3 μm, ($V < 0.1$ μm^3); rods, 0.5 × 0.3 μm ($V = 0.15$–0.5 μm^3)	–
5. *Chryseobacterium solincola*, str. NF4, NF5, facultative parasites	*Bacteroidetes*[3]	1.7[3]	Coccoids, 0.2–0.4 × 0.2–0.5 μm, ($V = 0.004$–0.02 μm^3); rods, 0.2 – 0.4 × 0.3–0.5 μm ($V = 0.004$–0.04 μm^3)	–
6. *Nanoarchaeum equitans*, obligate parasite	*Nanoarchaeota*[4]	0.58[4]	Cocci, $d \sim 0.4$ μm ($V < 0.1$ μm^3)	n.d.

N.d., no data.
[1] Data of Jurkevitch [49]; [2] data of Duda et al. [44]; [3] data of Suzina et al. [45]; [4] data of Huber et al. [16]; [5] data of Davidov et al. [50]; [6] data of Gromov and Mamkayeva [48].

The Phenomenon of Minimization

In microorganisms, there are two main manifestations of the minimization phenomenon: cell size minimization and genome size minimization.

Cell size reduction. This phenomenon is observed in two cases: in the course of ontogenetic development and aging of cultures of "usual" bacteria and in the course of evolutionary emergence of ultrasmall bacteria that are characterized by ultrasmall cells under normal conditions of active culture development (ultramicrobacteria, or nanobacteria). In old cultures of usual bacteria, small cells are formed as a result of the last reductive cell division in depleted media [8]. These cells are usually called filterable forms, since they pass through filters with a pore diameter of

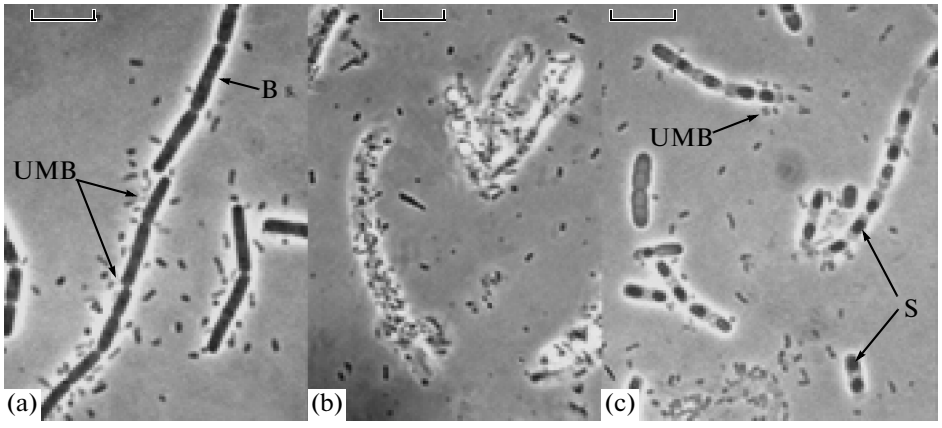

Fig. 3. Phase-contrast micrograph of a binary culture of *Bacillus subtilis* and *Chryseobacterium solincola*, strain NF4, after cultivation for 20 min (a) and for 7 days (b): parasitic UMB (NF4) cells attached to bacilli (a) and disintegrating bacillus cells surrounded with UMB (b), (c). Designations: UMB, NF4 cells; B, prey (*B. subtilis*) cells; S, spore. Bar, 10 μm.

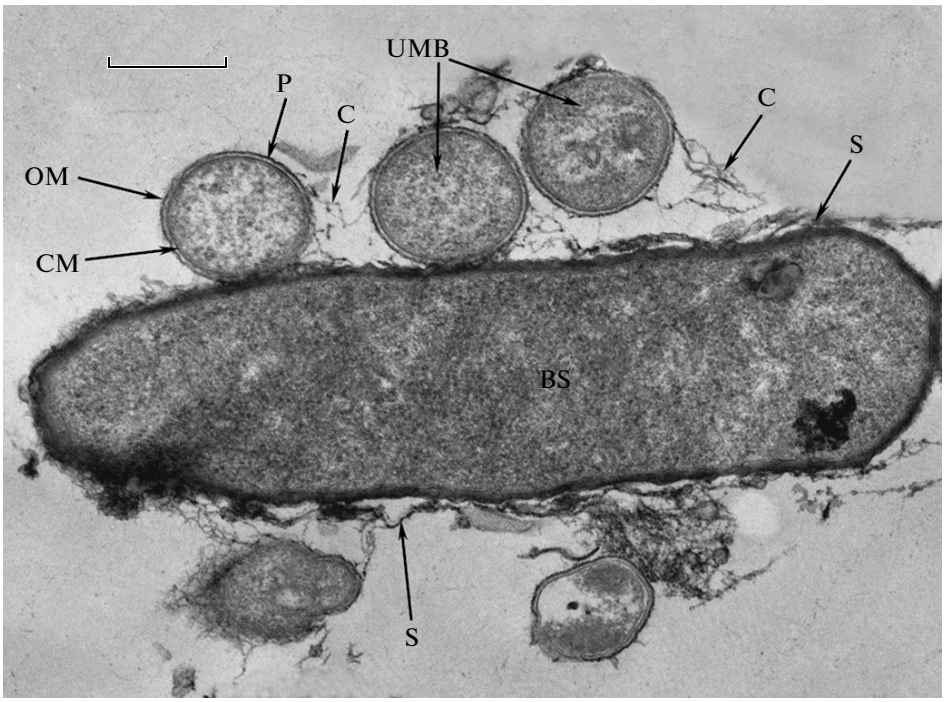

Fig. 4. Thin sections of cells of the parasitic UMB *Chryseobacterium solincola* strain NF4 adsorbed on cells of *Bacillus subtilis*. The NF4 cells are seen to attach to the S-layer of the *B. subtilis* cell envelope. Designations: UMB, NF4 cells; OM, outer membrane; C, capsule fibrils; P, periplasm; CM, cytoplasmic membrane; S, S-layer of the *B. subtilis* (BS) envelope. Bar, 0.3 μm.

0.2 μm. These cells have been poorly studied, but their size reduction is obviously associated, solely or primarily, with the reduction of the cytoplasmic component. Ultramicrobacteria are of greater interest in the evolutional and theoretical respects: their ultrasmall cell size is most often associated with genome size reduction.

Genome size minimization. It is known that reduction of prokaryotic genomes is most pronounced in intracellular symbionts. Thus, the genome of the endosymbiotic bacterium *Carsonella* consists of only 159662 base pairs, while the genome size of a large virus (mimivirus) is 1200000 base pairs. The data on the size and properties of the genomes of free-living saprotrophic UMB are of great significance because they make it possible (a) to estimate the direction and succession of the evolution of not only genomes but also of UMB as organisms; (b) to reveal the genes without which the existence of free-living UMB is impossible; (c) to establish the character of genome streamlining and the dependence of the cell size on the ratio between the cytoplasmic and nuclear components; (d) to choose a particular organism as a model or an object to be used in biotechnology; and (e) to find out which genes determine predation (parasitism) in UMB.

However, the genome size data have so far been obtained only for members of four species of free-living saprotrophic UMB: *Pelagibacter ubique* [26], *Sphingopyxis alaskensis* [24], and *Kaistia adipata* and *Chryseobacterium solincola* [45]. The most interesting data were obtained in the study of the *P. ubique* genome (only 1.3 Mb in size): it is ultimately rational, contains no nonworking or doubling genes, and lacks pseudogenes, introns, transposons, and extrachromosomal elements. The whole code is aimed at performance of nutrition as the main function. However, since there are no data on the genomes of most of the other UMB species, the general pattern of the peculiarities of structural and functional organization of UMB genomes is still far from being clear. Therefore, the question about the minimum permissible cell size for free-living saprotrophic UMB is still unanswered.

UMB Evolution: Are the Cultivated UMB Species Primordial Creatures?

This problem cannot be considered on the basis of scarce observations of virtual nanobacteria described in geological works. As for the cultivated species of ultramicrobacteria, the currently available data suggest that cultivated UMB do not have a common origin, and their taxa are mosaically distributed over the phylogenetic 16S rRNA tree of bacteria and archaea. Cultivated UMB have been found in seven large phylogenetic groups: *Alpha-, Beta-* and *Gammaproteobacteria*, *Bacteroidetes, Verrucomicrobiae, Actinobacteria*, and *"Elusimicrobia"*. Their closest phylogenetic relatives are species with larger cells and broader metabolic capacities. The origin of cultivated UMB by way

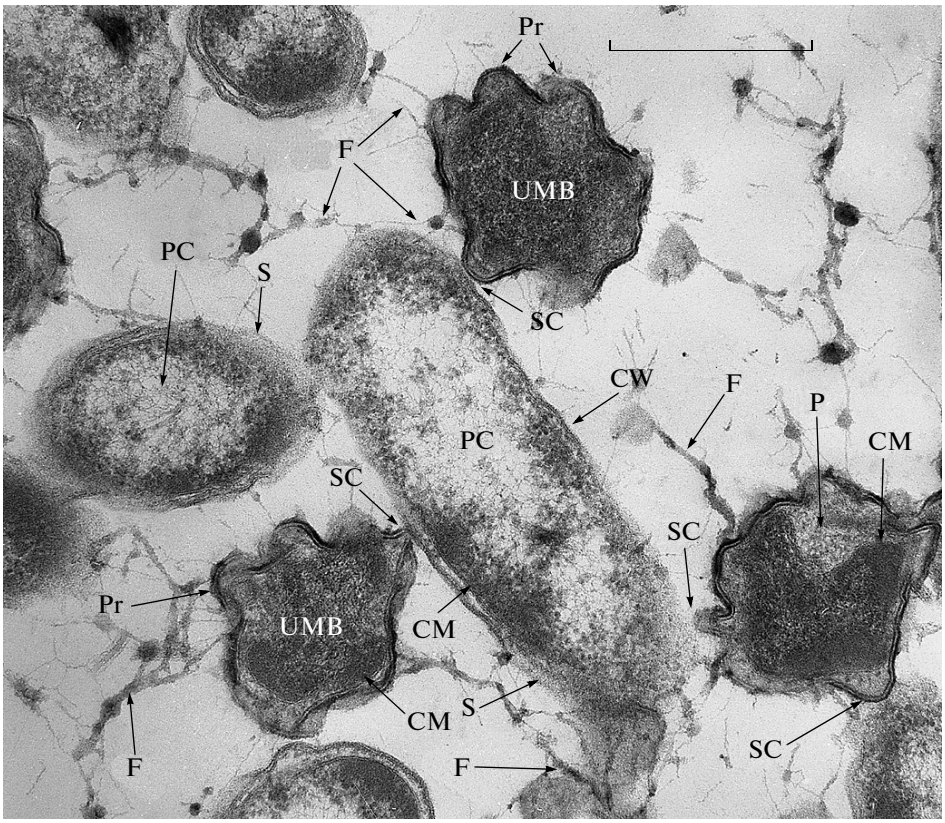

Fig. 5. Interaction between the parasitic UMB *Kaistia adipata* NF1 and the prey *Acidovorax delafieldii*. The sites of contacts between the protrusions of NF1 cells and the S-layer of the *A. delafieldii* cell envelope are shown. Electron micrograph. Designations: F, polysaccharide filaments; SC, sites of contact between UMB cell protrusions and *A. delafieldii* cell envelope; PC, *A. delafieldii* prey cell; UMB, NF1 cells; P, periplasm; Pr, protrusions of NF1 cells; CM, cytoplasmic membrane; CW, cell wall; S, S-layer of the envelope. Bar, 0.3 μm.

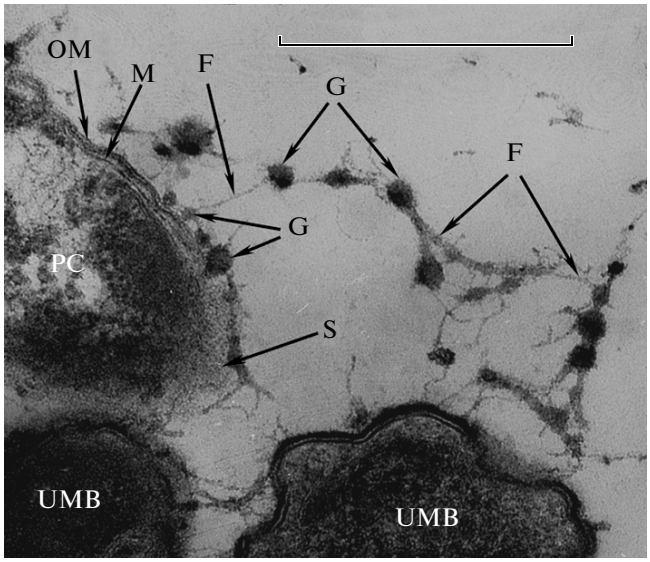

Fig. 6. Enlarged fragment of a thin section of interacting cells of the parasitic UMB *Kaistia adipata* NF1 and the prey *Acidovorax delafieldii*. The cells were stained with ruthenium red. Polysaccharide filaments attached to the envelope of the prey and globules on the filaments can be seen. Designations: S, tangential section of the S-layer; M, murein layer of the cell wall; OM, outer membrane; PC, prey cell; UMB, NF1 cells; F, polysaccharide filaments; G, adhesive granules on the filaments. Bar, 0.3 μm.

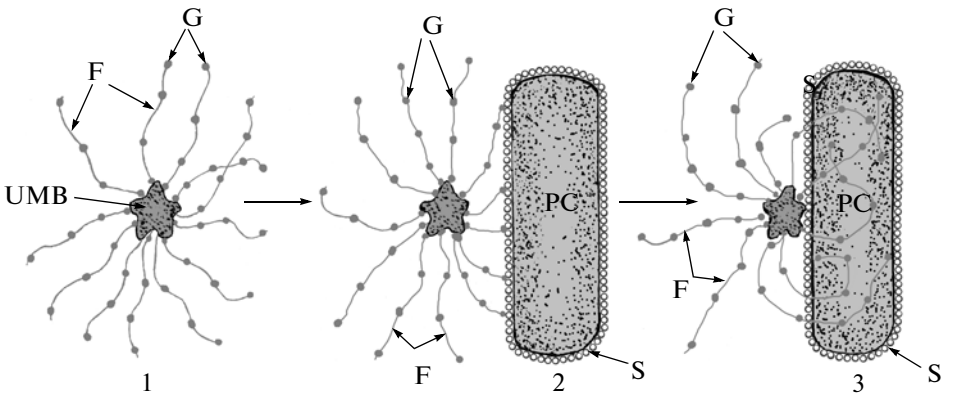

Fig. 7. Schematic representation of cohesion between the cells of parasitic UMB and their prey: 1, the initial stage: capture of the prey cell by polysaccharide filaments of the parasites; 2, the second stage: approach and convergence of interacting cells due to Brownian movement forces and due to gradual adhesion of new granules to the envelope surface; 3, the third stage; tight attachment of the cells. Designations: UMB, parasitic cell; PC, prey cell; F, polysaccharide filaments (strands); G, adhesive granules on the filaments; S, the surface S-layer of the prey cell.

of reductive evolution is also demonstrated by the data on adaptive decrease (shortening) and rationalization of the genome (e.g., in the free-living *"Pelagibacter ubique"*), large-scale genome reduction in parasitic mycoplasmas, narrowing of the range of utilizable substrates in oligotrophic species, and adaptation to parasitism of some species. Therefore, the currently cultivated UMB cannot be considered primordial forms of organisms.

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REFERENCES

1. Courties, C., Perasso, R., Chretiennot-Dinet, M.-J., Goui, M., Guillou, L., and Troussellier, M., Phylogenetic Analysis and Genome Size of *Ostreococcus tauri* (Chlorophyta, Prasinophyceae), *J. Physiol.*, 1998, vol. 34, pp. 844–849.
2. Matsuzaki, M., Misumi, O., Shin-I.T., et al., Genome Sequence of the Ultrasmall Unicellular Red Alga *Cyanidioschyzon merolae* 100, *Nature*, 2004, vol. 428, pp. 653–657.
3. Cavicchioli, R. and Ostrowski, M., Ultramicrobacteria, *Encyclopedia of Life Sciences*, 2003, www.els.net. pp. 1–8.
4. Lartique, C., Glass, J.I., Alperovich, N., Pieper, R., Parmar, P.P., Hutchison, C.A., III, Smith, H.O., and Venter, J.C., Genome Transplantation in Bacteria: Changing One Species to Another, *Science*, 2007, vol. 317, pp. 632–638.
5. Gibson, D.G., Lartique, C., Noskov, V.N., et al., Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome, *Science*, 2010, vol. 329, pp. 52–66.
6. Torrella, F. and Morita, R.Y., Microcultural Study of Bacterial Size Changes and Microcolony and Ultramicrocolony Formation by Heterotrophic Bacteria in Seawater, *Appl. Environ. Microbiol.*, 1981, vol. 41, pp. 518–527.
7. Bae, H.C., Cota-Robles, E.H., and Casida, L.E., Jr., Microflora of Soil as Viewed by Transmission Electron Microscopy, *Appl. Microbiol.*, 1972, vol. 23, pp. 637–648.
8. Velimirov, B., Nanobacteria, Ultramicrobacteria and Starvation Forms: A Search for the Smallest Metabolizing Bacterium, *Microbes and Environments*, 2001, vol. 16, pp. 67–77.
9. Schut, F., Prins, R.A., and Gottschal, J.C., Oligotrophy and Pelagic Marine Bacteria: Facts and Fiction, *Aquat. Microb. Ecol.*, 1997, vol. 12, pp. 177–202.
10. Panikov, N.S., Contribution of Nanosized Bacteria to the Total Biomass and Activity of a Soil Microbial Community, *Adv. Appl. Microbiol.*, 2004, vol. 57, pp. 245–296.
11. MacDonell, M.T. and Hood, M.A., Isolation and Characterization of Ultramicrobacteria from a Gulf Coast Estuary, *Appl. Environ. Microbiol.*, 1982, vol. 43, pp. 566–571.
12. Schut, F., Gottshal, J.C., and Prins, R.A., Isolation and Characterization of the Marine Ultramicrobacterium *Sphingomonas* sp. Strain RB2256, *FEMS Microbiol. Rev.,* 1997, vol. 20, pp. 363–369.
13. Dmitriev, V.V., Suzina, N.E., Rusakova, T.G., Gilichinskii, D.A., and Duda, V.I., Ultrastructural Characteristics of Natural Forms of Microorganisms Isolated from Permafrost Grounds of Eastern Siberia by the Method of Low-Temperature Fractionation, *Dokl. Biol. Sci.*, 2001, vol. 378, p. 304.

14. Dmitriev, V.V., Suzina, N.E., Barinova, E.S., Duda, V.I., and Boronin, A.M., An Electron Microscopic Study of the Ultrastructure of Microbial Cells in Extreme Biotopes in situ, *Microbiology*, 2004, vol. 73, no. 6, pp. 716–723.

15. Dmitriev, V.V., Suzina, N.E., Rusakova, T.G., Petrov, P.Yu., Oleinikov, R.R., Esikova, T.Z., Kholodenko, V.P., Duda, V.I., and Boronin, A.M., Electron Microscopic Detection and in situ Characterization of Bacterial Nanoforms in Extreme Biotopes, *Microbiology*, 2008, vol. 77, no. 1, pp. 39–46.

16. Huber, H., Hohn, M.J., Rache, R., Fuchs, T., Wimmer, V.C., and Stetter, K.O., A New Phylum of Archaea Represented by a Nanosized Hyperthermophilic Symbiont, *Nature*, 2002, vol. 417, pp. 63–67.

17. Baker, B.J., Comolli, L.R., Dick, G.J., Hauser, L.J., Hyatt, D., Dill, B.D., Land, M.L., VerBerkmoes, N.C., Hettich, R.L., and Banfield, J.F., Enigmatic, Ultrasmall, Uncultivated Archaea, *Proc. Natl. Acad. Sci. USA*, 2010, vol. 107, pp. 8806–8811.

18. Morita, R.Y., Bioavailability of Energy and Its Relationship to Growth and Starvation Survival in Nature, *Can. J. Microbiol.*, 1988, vol. 34, pp. 436–441.

19. Folk, R.L., Nannobacteria and the Precipitation of Carbonate in Unusual Environments, *Sediment. Geol.*, 1999, vol. 126, pp. 47–55.

20. Kajander, E.O. and Ciftcioglu, N., Nanobacteria—An Alternative Mechanism for Pathogenic Intra- and Extracellular Calcification and Stone Formation, *Proc. Natl. Acad. Sci. USA*, 1998, pp. 8274–8279.

21. Sieburth, J.M.N., Smetacek, V., and Lenz, J., Pelagic Ecosystem Structure: Heterotrophic Compartments of the Plankton and Their Relationship to Plankton Size Fractions, *Limnol. Oceanogr.*, 1978, vol. 23, pp. 1256–1263.

22. Haller, C.M., Röleke, S., Vybiral, D., Witte, A., and Velimirov, B., Investigation of 0.2 μm Filterable Bacteria from Western Mediterranean Sea Using a Molecular Approach: Dominance of Potential Starvation Forms, *FEMS Microbiol. Ecol.*, 2000, vol. 31, pp. 153–161.

23. Rappé, M.S., Connon, S.A., Vergin, K.L., and Giovannoni, S.J., Cultivation of the Ubiquitous SAR11 Marine Bacterioplankton Clade, *Nature*, 2002, vol. 48, pp. 630–633.

24. Giovannoni, S.J., Bibbs, L., Cho, J.-C., Staples, M.D., Desiderio, R., Vergin, K.L., Rappé, M.S., Laney, S., Barofsky, D.F., and Mathur, E., Proteorhodopsin in the Ubiquitous Marine Bacterium SAR11, *Nature*, 2005a, vol. 438, pp. 82–85.

25. Giovannoni, S.J., Tripp, H.J., and Givan, S., et al., Genome Streamlining in a Cosmopolitan Oceanic Bacterium, *Science*, 2005b, vol. 309, pp. 1242–1245.

26. Cavicchioli, R., Ostrowski, M., Fegatella, F., Goodchild, A., and Guixa-Boixereu, N., Life under Nutrient Limitation in Oligotrophic Marine Environments: An Ecophysiological Perspective of *Sphingopyxis alaskensis* (Formerly *Sphingomonas alaskensis*), *Microb. Ecology*, 2003, vol. 45, no. 3, pp. 203–217.

27. Miyoshi, T., Iwatsuki, T., and Naganuma, T., Phylogenetic Characterization of 16S rRNA Gene Clones from Deep-Groundwater Microorganisms that Pass through 0.2-Micrometer-Pore-Size Filters, *Appl. Environ. Microbiol.*, 2005, vol. 71, pp. 1084–1088.

28. Silbaq, F.S., Viable Ultramicrocells in Drinking Water, *J. Appl. Microbiol.*, 2009, vol. 106, pp. 106–117.

29. Miteva, V.I. and Brenchley, J.E., Detection and Isolation of Ultrasmall Microorganisms from a 120000-Year-Old Greenland Glacier Ice Core, *Appl. Environ. Microbiol.*, 2005, vol. 71, pp. 7806–7818.

30. Loveland-Curtze, J., Miteva, V., and Brenchley, J., Novel Ultramicrobacterial Isolates from a Deep Greenland Ice Core Represent a Proposed New Species, *Chryseobacterium greenlandense* sp. nov., *Extremophiles*, 2010, vol. 14, no. 1, pp. 61–69.

31. Loveland-Curtze, J., Miteva, V.I., and Brenchley, J.E., *Herminiimonas glaciei* sp. nov., a Novel Ultramicrobacterium from 3042 M Deep Greenland Glacial Ice, *Int. J. Syst. Evol. Microbiol.*, 2009, vol. 59, pp. 1272–1277.

32. Balkwill, D.L. and Casida, L.E., Microflora of Soil as Viewed by Freeze-Etching, *J. Bacteriol.*, 1973, vol. 114, pp. 1319–1327.

33. Janssen, P.H., Schuhmann, A., Möschel, E., and Rainey, F.A., Novel Anaerobic Ultramicrobacteria Belonging to the *Verrucomicrobiales* Lineage of Bacterial Descent Isolated by Dilution Culture from Anoxic Rice Paddy Soil, *Appl. Environ. Microbiol.*, 1997, vol. 63, pp. 1382–1388.

34. Iizuka, T., Yamanaka, S., Nishiyama, T., and Hiraishi, A., Isolation and Phylogenetic Analysis of Aerobic Copiotrophic Ultramicrobacteria from Urban Soil, *J. Gen. Appl. Microbiol.*, 1998, vol. 44, pp. 75–84.

35. Sahin, N., Gonzalez, J.M., Iizuka, T., and Hill, J.E., Characterization of Two Aerobic Ultramicrobacteria Isolated from Urban Soil and a Description of *Oxalicibacterium solurbis* sp. nov., *FEMS Microbiol. Lett.*, 2010, vol. 307, pp. 25–29.

36. Rutz, B.A. and Kieft, T.L., Phylogenetic Characterization of Dwarf Archaea and Bacteria from a Semiarid Soil, *Soil Biol. Biochem.*, 2004, vol. 36, pp. 825–833.

37. Lysak, L.V., Lapygina, E.V., Konova, I.A., and Zvyagintsev, D.G., Quantity and Taxonomic Composition of Ultramicrobacteria in Soils, *Microbiology*, 2010, vol. 79, no. 3, p. 408.

38. Urbano, P. and Urbano, F., Nanobacteria: Facts or Fancies?, *PLoS Pathog.*, 2007, vol. 3, no. 5, e55, pp. 0567–0570.

39. Cisar, J.O., Xu, D.Q., Thompson, J., Swaim, W., Hu, L., and Kopecko, D.J., An Alternative Interpretation of Nanobacteria-Induced Biomineralization, *Proc. Natl. Acad. Sci. USA*, 2000, vol. 97, pp. 11511–11515.

40. Hahn, M.W., Lunsdorf, H., Wu, Q., Shauer, M., Hofle, M.G., Boenigk, J., and Stadler, P., Isolation of Novel Ultramicrobacteria Classified as Actinobacteria from Five Freshwater Habitats in Europe and Asia, *Appl. Environ. Microbiol.*, 2003, vol. 69, pp. 1442–1451.

41. May, H.D., Miller, G.S., Kjellerup, B.V., and Sovers, K.R., Dehalorespiration with Polychlorinated Biphenyls by an Anaerobic Ultramicrobacterium, *Appl. Environ. Microbiol.*, 2008, vol. 74, pp. 2089–2094.

42. Geissinger, O., Herlemann, D.P.R., Mörshel, E., Maier, U.G., and Brune, A., The Ultramicrobacterium *"Elusimicrobium minutum"* gen. nov., sp. nov., the First

Cultivated Representative of the Termite Group 1 Phylum, *Appl. Environ. Microbiol.*, 2009, vol. 75, pp. 2831–2840.

43. Herlemann, D.P.R., Geissinger, O., Ikeda-Ohtsubo, W., Kunin, V., Sun, H., Lapidus, A., Hugenholtz, P., and Brune, A., Genomic Analysis of *"Elusimicrobium minutum"*, the First Cultivated Representative of the Phylum *"Elusimicrobia"* (Formerly Termite Group 1), *Appl. Environ. Microbiol.*, 2009, vol. 75, pp. 2841–2849.
44. Duda, V.I., Suzina, N.E., Esikova, T.Z., Akimov, V.N., Oleinikov, R.R., Polivtseva, V.N., Abashina, T.N., Shorokhova, A.P., and Boronin, A.M., A Cytological Characterization of the Parasitic Action of Ultramicrobacteria NF1 and NF3 of the Genus *Kaistia* on Chemoorganotrophic and Phototrophic Bacteria, *FEMS Microbiol. Ecol.*, 2009, vol. 69, pp. 180–193.
45. Suzina, N.E., Duda, V.I., Esikova, T.Z., Shorokhova, A.P., Gafarov, A.B., Oleinikov, R.R., Akimov, V.N., Abashina, T.N., Polivtseva, V.N., and Boronin, A.M., Novel Ultramicrobacteria, Strains NF4 and NF5, of the Genus *Chryseobacterium*: Facultative Epibionts of *Bacillus subtilis, Microbiology*, 2011, vol. 80, no. 4, pp. 535–548.
46. Young, K.D., The Selective Value of Bacterial Shape, *Microbiol. Mol. Biol. Rev.*, 2006, vol. 70, pp. 660–703.
47. Lambina, V.A., Afinogenova, A.V., Romai Penabad, S., Konovalova, S.M., and Pushkareva, A.P., *Micavibrio admirandus* gen. et sp. nov., *Mikrobiologiya*, 1982, vol. 51, pp. 114–117.
48. Gromov, B.V. and Mamkaeva, K.A., Proposal of the New Genus *Vampirovibrio chlorellavorus* for a Bacterium Formerly Assigned to *Bdellovibrio, Mikrobiologiya*, 1980, vol. 49, pp. 165–167.
49. Jurkevitch, E., Predatory Behaviors in Bacteria—Diversity and Transitions, *Microbe*, 2007, vol. 2, pp. 67–73.
50. Davidov, Y., Huchon, D., Kova, S., and Jurkevitch, E., A New α-Proteobacterial Clade of *Bdellovibrio*-like Predators: Implications for the Mitochondrial Endosymbiotic Theory, *Environ. Microbiol.*, 2006, vol. 8, pp. 2179–2188.