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EXPERIMENTAL ARTICLES

Ultrastructural Organization and Development Cycle of Soil Ultramicrobacteria Belonging to the Class *Alphaproteobacteria*

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Abstract—Gram-negative chemoorganotrophic soil ultramicrobacteria (UMB), strains NF1 and NF3, have been isolated. In their development cycle, the strains formed small coccoid cells of 400–800 nm and ultrasmall cells of 200–300 nm. Phylogenetically, the strains NF1 and NF3 belong to *Alphaproteobacteria* and are close to the type strain of the recently described species *Kaistia adipata*. The ultrastructure of UMB cells has been studied using ultrathin sections and freeze-fracturing. It has been shown that the structure of UMB cell walls is of the gram-negative type; the outer membrane and peptidoglycan layer are well differentiated. The cell surface has numerous protrusions (prosthecae) of conical or spherical shape filled with the contents of the periplasm. The formation of unusual cellular structures (not occurring in known free-living bacteria) is a feature of UMB; these include the following: (a) piles of rod-like subunits, ca. 30 Å in diameter and 150–250 Å in length; (b) long bunches (up to 300–400 Å) comprised of filamentous subunits; and (c) large electron-dense spherical bodies (up to 200–300 Å in diameter) localized in the periplasm. A distinctive feature of UMB is their ability to grow as facultative parasites on living cyanobacterial (CB) cells. In this case, three types of interaction between UMB and CB have been revealed: (1) adsorption of UMB cells on the surface of CB cells; (2) penetration of UMB into polysaccharide sheathes; and (3) penetration of UMB into CB cytoplasm. UMB cells have been shown to reproduce by budding, with buds (up to 2–3) located directly on the mother cell, without formation of intermediate hyphae.

Key words: ultramicrobacteria, cell ultrastructure, parasitism, cell–cell interaction, electron and fluorescence microscopy, binary bacterial cultures.

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The study of ultramicrobacteria (UMB) inhabiting seas and intracontinental water bodies is reported in the works of many researchers [1–4]; however, only a few works concern the isolation and characterization of soil UMB [3, 5–7]. These works report the obtaining of soil UMB isolates with the species status as yet unspecified.

The isolation and characterization of genotypical and phenotypical properties of pure cultures is essential in order to assess the role of UMB in the biosphere, their ultrastructural and metabolic diversity, and the probability of as yet unknown biochemical processes occurring in their cells. In situ investigation of microorganisms in oil slimes by the methods of transmission electron microscopy and ultrathin sections of soil micromonoliths [8] has revealed an unusual ultrasmall bacterium (nanobacterium). A large fraction of its coccoid cells have nanometer dimensions: their diameter is 0.2–0.3 μm, or even below 0.2 μm in some cells; thus, they are not visible under a common light microscope.

This paper is devoted to the isolation of an ultramicrobacterium similar to the nanobacterium that we found in oil slime in situ; its cytology and development cycle are characterized.

MATERIALS AND METHODS

Substrates for the isolation of bacteria. The following substrates were used: (1) oil slime (slime enriched with oil products) that had stayed under field conditions for over 35 years (Nizhnekamsk, Tatarstan, Russia), containing up to 40% of organic compounds (ca. 30% of these were oil products); (2) light chestnut

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soil, horizon B_1 (Prikaspiyskaya lowland, Volgograd oblast, Russia); (3) benthic silt of Lake Baikal; (4) laboratory water culture of the plant *Pedilantus tithymaloides* (family *Euphorbiacea*).

Media and methods for the isolation and characterization of physiological properties and taxonomic characteristics of the bacteria. The bacteria were primarily isolated and their pure cultures were obtained using the following media: (a) agarized oil slime (AO): crude oil-slime, 30 g; agar, 6 g; and tap water, 300 ml; (b) agarized soil (AS): air-dried humus–gley soil (horizon A_1) (Prioksko–Terrasny Nature Reserve) or light chestnut soil (horizon B_1), 30 g; agar, 6 g; and water, 300 ml (sterilized at 1 atm for 1 h); and (c) tryptone–soy agar (medium 5/5 prepared at IBPM, Russian Acad. Sci.) for isolation of bacteria from the near-root zone of *P. tithymaloides*). The isolates were phenotypically characterized by the standard methods [9].

Cyanobacterial cultures. The collection cyanobacterial strains used in the study included the following: *Chlorogloeopsis fritschii* ATCC 27193; *Anabaena variabilis* ATCC 29413; *Nostoc muscorum* 11; and *Spirulina* sp. 287; as well as *Chlorogloeopsis* sp. S, which had been isolated from oil slime and identified to the genus level. Cyanobacteria were grown in a liquid or agarized BG 11 medium [10] at 28°C under continuous illumination with luminescent lamps (1500 lx).

Growth of co-cultures. Liquid cyanobacterial cultures grown for 20 days were supplemented with equal volumes of liquid five-day cultures of strains NF1 and NF3 incubated in a synthetic medium with glucose and containing ca. ~3–3.8×10^{10} CFU/ml. Cultivation was carried out at 20°C. The observations were performed for 30 days.

Biochemical and molecular biological methods. The qualitative composition of cytochromes in the cells of the isolates was determined from the differential absorbance spectra and the first-derivative spectra according to [11] in a Shimadzu UV-160 spectrophotometer. The presence of plasmid DNA in the cells was tested by a modified method of Eckhardt [12]. The DNA mol.% G+C content was determined by thermal denaturing [13].

Analysis of the nucleotide sequence of the 16S rRNA gene. The 16S rRNA genes were amplified using PCR and universal bacterial primers 27F and 1492R [14] and sequenced in a Beckman–Coulter CEQ™ 2000XL DNA Analysis System. The nearly complete 16S rRNA gene sequence was used for phylogenetic analysis with TREECON [15].

Microscopy methods. The morphology and thin structure of bacterial cells were studied by phase contrast, epifluorescence, and electron microscopy. For epifluorescence microscopy, the cells in co-cultures of NF1, NF3, and cyanobacteria were fixed with 1.5% glutaraldehyde for 30 min and stained 1 μg/ml with DAPI (4,6-diamidino-2-phenylindole) for 5 min. The preparations were examined with Polyvar (Reichert) and LUMAM (LOMO, Russia) microscopes under UV excitation with the maximum at 360 nm. DAPI staining revealed NF1, NF3, and cyanobacterial cells concurrently. Cyanobacteria were visible due to their autofluorescence: their red and orange fluorescence was induced by the presence of chlorophyll and phycobilins in their cells; in addition, their sheaths also exhibited weak green fluorescence.

Replicas of cell freeze-fractures were obtained in a JEE-4X vacuum assembly using devices that provided the cooling of microbial cells at a rate of ca. ~10^6 deg/sec. The biomass was frozen in liquid propane supercooled with liquid nitrogen to –196°C. Freeze-fracture was made under vacuum (3×10^{-4} Pa) at the sample temperature of –100°C. The replica from the freeze-fracture surface was obtained by applying the platinum–carbon mixture at an angle of 30° and the strengthening layer of pure carbon at an angle of 90° under vacuum.

Oil-slime micromonoliths and cell biomass were used to prepare ultrathin sections. Micromonoliths were obtained from a 10-kg sample of weakly humid oil slime that had been maintained in a stationary condition for 3–6 months. A cube with a ca. 3–5-mm side was cut out with a sterile scalpel and lancet so as not to disturb the natural composition of the material. Micromonoliths and cell biomass of pure cultures were prefixed with 1.5% (vol/vol) glutaraldehyde solution in a 0.05 M cacodylate buffer (pH 7.2) at 4°C for 1 h. After three washings with the same buffer, the material was additionally fixed with 1% OsO_4 in 0.05 M cacodylate buffer at 20°C for 3 h. To contrast the cell wall polysaccharides, sheaths, and capsule substances, glutaraldehyde–osmium fixation in the presence of ruthenium red was applied [16]. After dehydration, the material was embedded into Epon 812, and ultrathin sections were made on an LKB III ultratome (Sweden). The sections were mounted on copper grids covered with Formvar film, contrasted with uranyl acetate (3% solution in 70% ethanol) for 30 min, and then stained with lead citrate [17] at 20°C for 4–5 min. Preparations of intact bacterial cells were negatively contrasted with a 0.2% aqueous solution of uranyl acetate. The preparations were examined under a JEM-100B electron microscope at 60 kV accelerating voltage.

RESULTS

Detection and isolation of ultramicrobacteria. Cell morphology. To study indigenous microorganisms in situ by transmission electron microscopy, oil-slime samples as micromonoliths were fixed and embedded into the epoxy resin to obtain ultrathin sections. Due to the high content of organic matter and minor share of solid mineral particles, it was possible to obtain ultrathin sections of good quality. Electron microscopic examination revealed six different morphotypes of ultramicrobacteria, including an unusual bacterium with coccoid cells of ca. 0.2–0.3 μm in diameter; some bacteria were less than 0.2 μm in diameter,

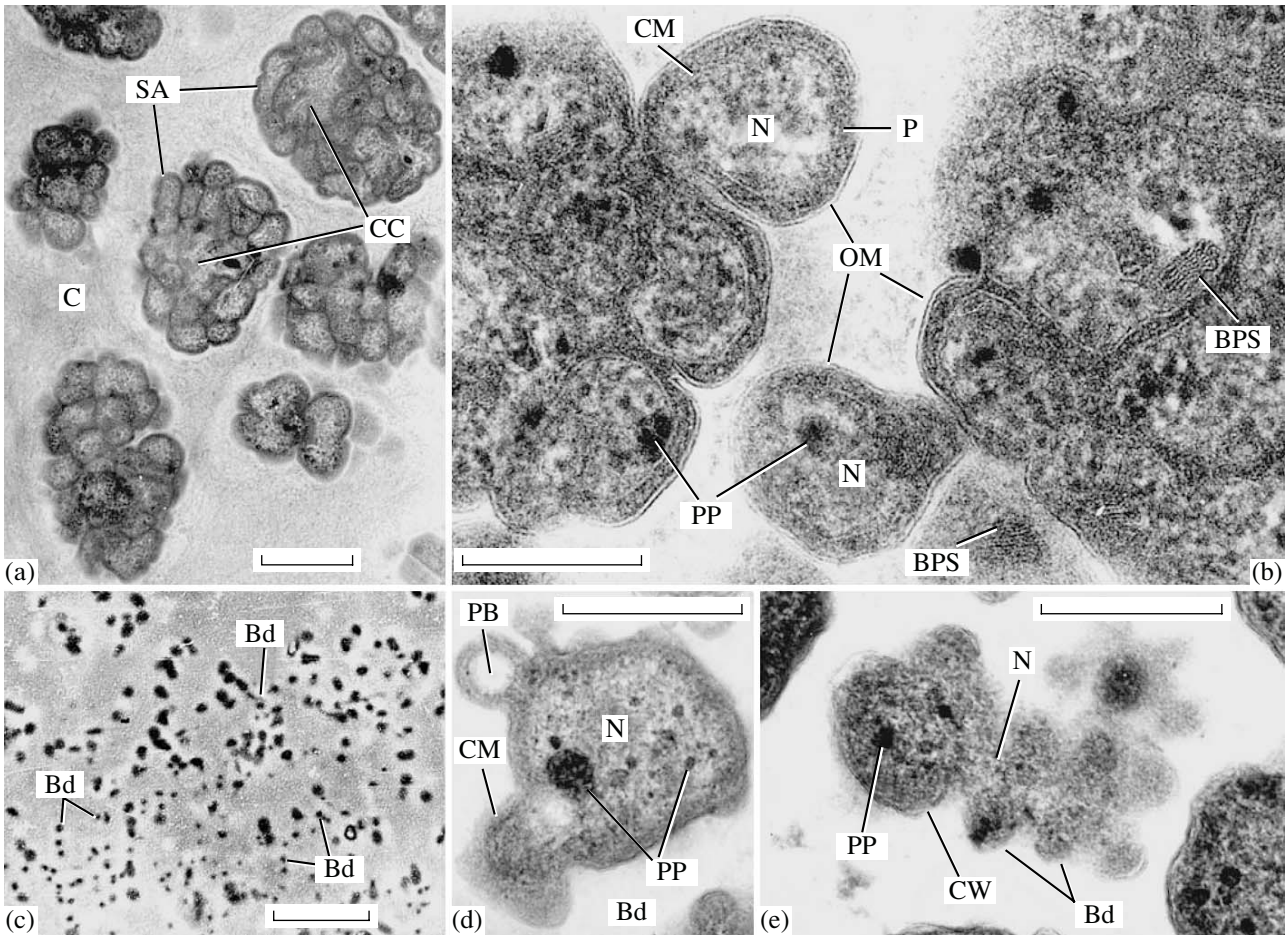

Fig. 1. Electron microscopic (a, b, d, e) and phase contrast (c) microphotographs of NF1 cells: (a), ultrathin section of aggregates of nanocells as spherical aggregates (SA) localized as microcolonies in an oil-slime sample; (b), enlarged fragment of the peripheral part of two SA. Thin structure of SA compartments and spherical nanocells branching from SA can be seen; (c), cells under a phase contrast microscope. Nanocells in chains and clusters can be seen (four-day culture on AO); (d), (e), thin structure of NF1 cells from colonies on AO (two-day culture); Figure 1d shows the cytoplasm passing into a bud. Scale bar in Figs. 1a, b, d, e, 300 nm; Fig 1c, 10 μm. Designations (Figs. 1–8): OMV, outer membrane vesicles; Gr, granular substance in the periplasm; OM, outer membrane; C, capsular layer; Cy, cytoplasm; CW, cell wall; N, nucleoid; P, periplasm; Bd, bud; BF, bunches of filaments; BPS, bodies with periodical structure; PB, poly-β-hydroxybutyrate granules; Cr, constriction; PP, polyphosphate granules; CC, central cell; CM, cytoplasmic membrane; SA, spherical aggregates of nanocells; SB, spherical bodies in the periplasm; SP, spherical protrusions.

i.e., could not be distinguished under a conventional light microscope. These cells form spherical aggregates (clusters) (Fig. 1a, 1b). Analysis of numerous spherical aggregates (SA) revealed that most individuals in them were at the stage of division, and that a fraction of small peripheral cells separated from other cells and were released into the medium after the formation of their envelopes had been completed (Fig. 1b). On completion of maturation, SA disintegrated into single cells or elements consisting of two to four cells.

Cell aggregates were visible under phase contrast as spherical bodies with granular structure, up to 4–6 μm in diameter. This fact made it possible to reveal them in the preparations and to transfer them from cover glasses to the AO medium surface. An enrichment culture was thus obtained; repeated transfers on AO enabled us to obtain a pure culture designated as strain NF1 (Figs. 1c–1e, 2a–2c). The culture purity was controlled by (a) its inoculation in various nutrient media: synthetic medium supplemented with different carbohydrates, LB, tryptone–soy, Ashby, BG-11, and AO, with verification of the absence of growth of contaminating microorganisms; and (b) electron microscopy of negatively contrasted whole cell preparations. The typical diagnostic characteristics of strain NF1 (apart from the small cell size) are as follows: (1) the presence of cells with the surface covered by spherical or conical protrusions (Fig. 2c); (2) the presence of spherical cell aggregates (Fig. 1e) similar to the SA observed in situ (Fig. 1a); (3) the presence of budding cells (Fig. 2b); and (4) the formation of typical rounded, wax-like, convex, slimy colonies, 2–3 mm in diameter on AO and up to 5–6 mm on synthetic media with carbohydrates. Using these characters as diagnostic ones, one more

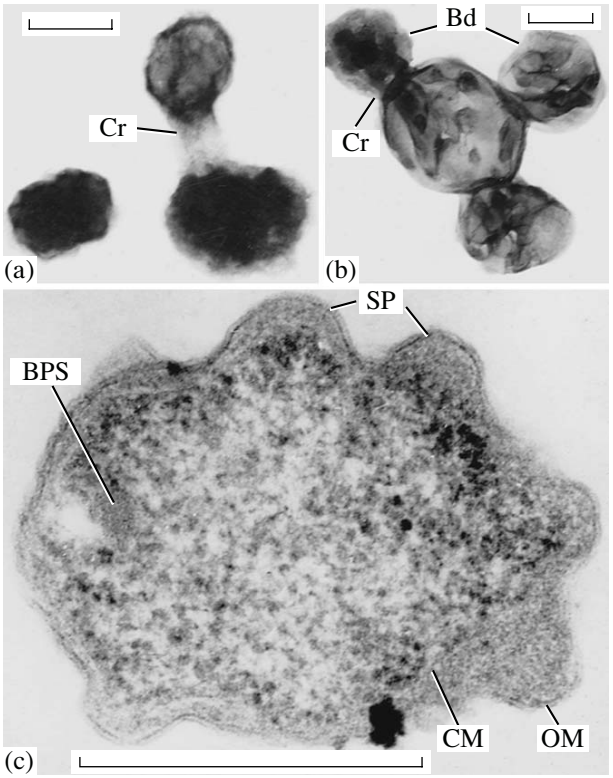

Fig. 2. NF1 cells: a, dividing nanocells; b, budding cell (intact negatively contrasted cells); c, ultrathin section of a cell from two-day culture on AO. Scale bar: Figs. 2a and 2b, 0.3 μm; Fig. 3c, 0.5 μm. Designations are as in Figure 1.

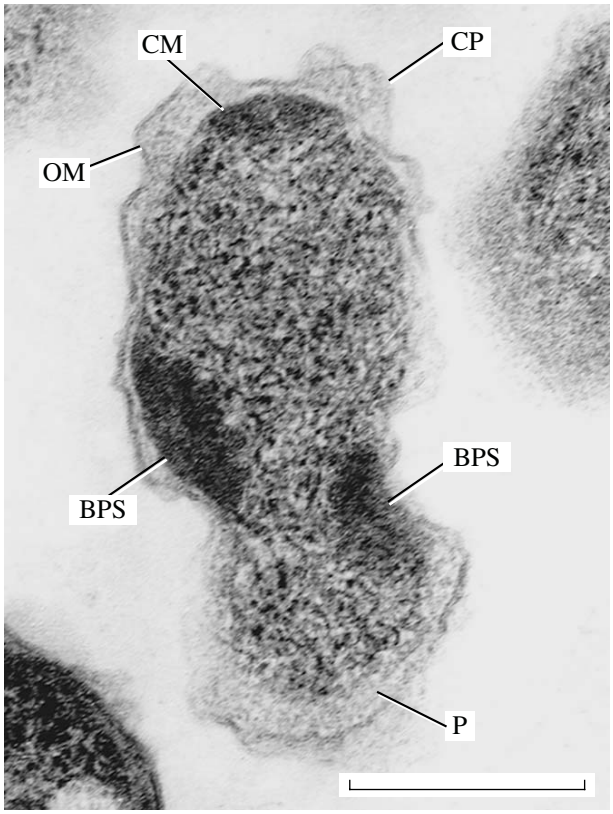

Fig. 3. Ultrathin section of a NF3 cell from a two-day culture grown on 5/5 agarized medium . Black regions in the cytoplasm: bodies with periodical structure (BPS). Scale bar is 0.5 μm. Designations are as in Figure 1.

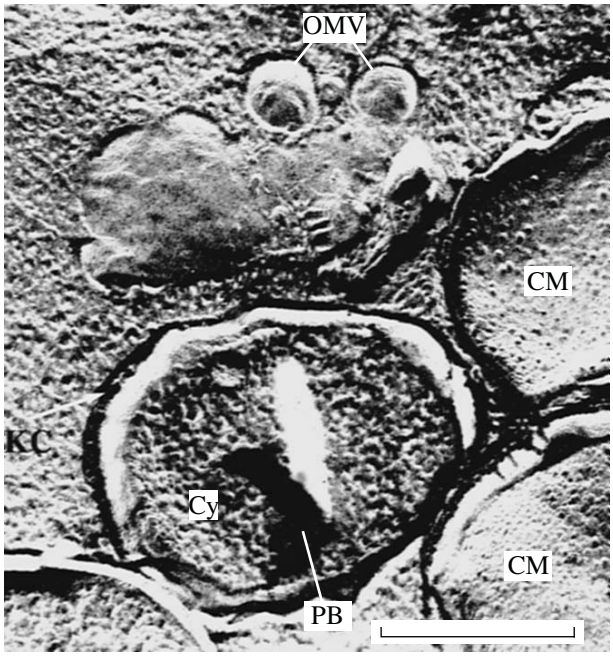

Fig. 4. Freeze-fracture of cells from a two-day culture of NF1 grown on AO. A conical body formed by PB and outer membrane vesicles (OMV) can be seen. Scale bar is 0.5 μm. Designations are as in Figure 1.

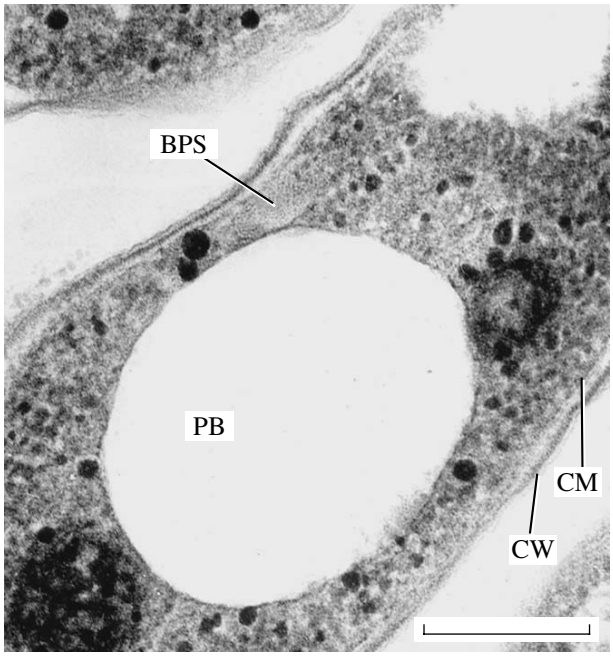

Fig. 5. Ultrathin section of a NF1 cell. BPS and PB are shown. Scale bar is 0.2 μm. Designations are as in Figure 1.

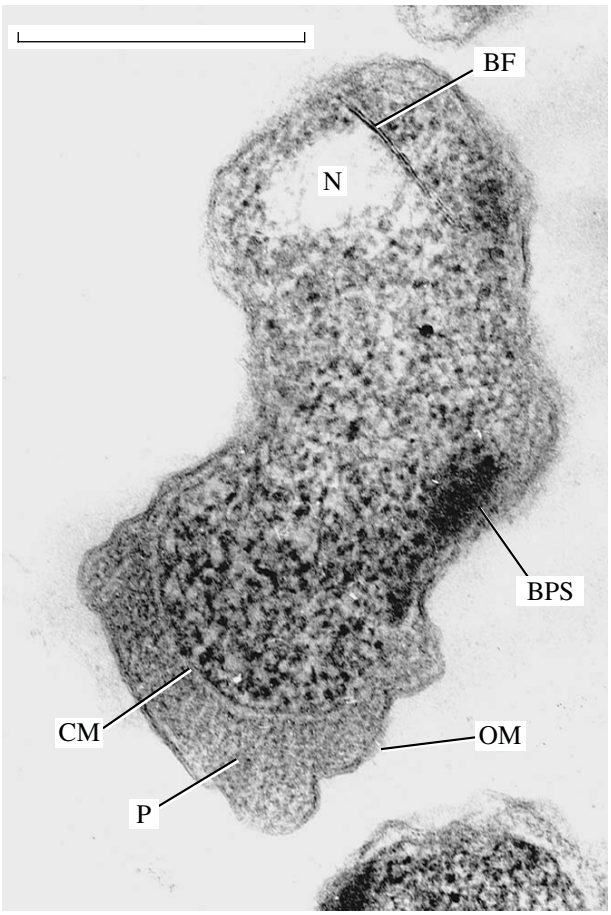

Fig. 6. Ultrathin section of a NF3 cell. BF and BPS are shown. Scale bar is 0.5 μm. Designations are as in Figure 1.

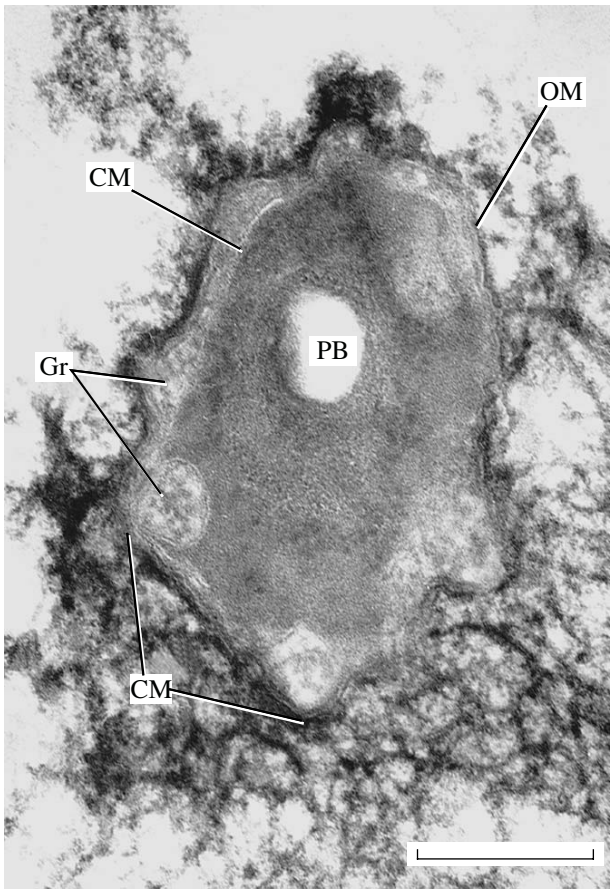

Fig. 7. Ultrathin section of a NF1 cell from a three-day co-culture with CB *Chlorogloeopsis fritschii*. The UMB cell is submerged into the capsular layer of the cyanobacterial cell. Granular substance (Gr) in rounded periplasmic pockets and conical protrusions on the cell can be seen as well. The cells are stained with ruthenium red. Scale bar is 0.3 μm.

pure culture was obtained: strain NF3 with the properties analogous to strain NF1 (Figs. 3, 6). This strain was isolated by inoculation of washout from the roots of *Pedilanthus tithymaloides* water culture on AS medium.

On synthetic media with carbohydrates, strains NF1 and NF3 form two types of cells: ultrasmall coccoid (rarely rod-shaped) cells, ca. ~300 nm (0.3 μm) in diameter, and large oval cells, 400 × 800 nm (0.4–0.8 μm) (Figs. 1–8, 11). The organisms reproduce by budding; the buds (one to three per cell) are located directly on the mother cells with no hyphae formed between them. Two to three buds are formed simultaneously on a single mother cell (Fig. 2b). Figure 9 presents four versions of the cell cycle. Figure 2a shows the fifth version of division of ultrasmall cells, particularly typical of old (1–2-month) cultures grown on AS (Figs. 1c, 2a). It should be noted that ultrasmall cells are able to pass through membrane filters (Millipore) with pore diameter 0.2 μm and to reproduce and grow upon their transfer to nutrient media. Many of the ultrasmall cells formed in old cultures can persist for a long time (5–6 months) without reproduction. Considering the formation of ultrasmall cells in the cycle of culture development, the isolated strains may be defined as ultramicrobacteria (UMB).

Cell ultrastructure. The ultrathin sections of cells show that the cell walls of strains NF1 and NF3 refer to the gram-negative type of structure, which is evident from the presence of an outer membrane (OM), periplasmic space (PS), and murein layer (ML) (Figs. 1b, d, e; 2c, 3, 5–7) located between the OM and the cytoplasmic membrane (CM). The KOH test also confirmed the affiliation of strains NF1 and NF3 with gram-negative bacteria.

Cell surface. The cell wall (CW) is covered with a thin layer of microcapsule (C), which is particularly expressed in co-cultures with cyanobacteria (Fig. 11). Flagella and pili are absent.

The spherical and conical protrusions (SP) on a cell surface are local extensions of the periplasmic zone (Figs. 2c, 3, 7) and, therefore, can be designated as periplasmic prosthecae. It should be noted that the murein layer in the places of SP localization is not revealed in

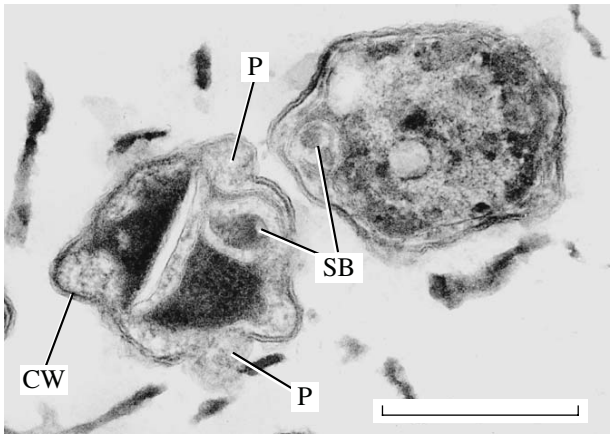

Fig. 8. Ultrathin section of NF1 cells from a three-day coculture with CB *Chlorogloeopsis fritschii*. Spherical bodies (SB) can be seen in the periplasm. The cells are stained with ruthenium red. Scale bar is 0.5 μm. Designations are as in Figure 1.

the sections. SP are the sites of formation of buds or extracellular vesicles surrounded by OM (Figs. 4, 11), as well as of the contact with the surface of cyanobacterial cells.

Cytoplasm. The cytoplasm has a well discernable nucleoid zone and inclusions of reserve compounds: poly-β-hydroxybutyrate (PB) and polyphosphates (PP). On ultrathin sections, PB appeared as spherical electron-transparent bodies surrounded by a thin single-layered wall, ca. ~30 Å thick (Figs. 1d, 5, 7); on freeze-fracture replicas, they were observed as round plates with the central part extended upwards as sharp-topped cones (Fig. 4). It is known that only bacterial PB granules form such cone-like artifact structures on freeze-fractures. PP on ultrathin sections usually appeared as spherical electron-dense bodies with a granular structure, localized in the cytoplasm and nucleoplasm; sometimes, however, their central part contained an electron-transparent core (Figs. 1d, 2c, 5). The cytoplasm shows unique crystal-like bodies with a periodical structure (BPS) (Figs. 1b, 2c, 3, 5, 6); these are piles of densely packed rod-shaped subunits, ca. ~30 Å thick and up to 150–300 Å long. These are most probably protein structures: they are well stained with lead salts and often appear as electron-dense sites within the cell (Figs. 4–6). Strain NF3 has two to three BPS per section of the central part of a cell (Fig. 3), while strain NF1 has only one BPS (Figs. 2c, 6). The only ultrastructural analog of BPS described in the literature is the terminal organelle of *Mycoplasma pneumoniae*, which also exhibits structural periodicity [18, 19]. All these inclusions (except for PB) are also present in the cells of spherical aggregates observed in situ in oil slime (Figs. 1, 2). Bunches of long (up to 50–60 nm) filaments (BF), ca. ~35–40 Å thick (Fig. 6) are another unusual structure occurring in the cells of strain NF3. The functions of BPS and BF are still unknown. One more unique ultrastructural peculiarity of the isolated bacterium is a marked structural differentiation of the periplasm, a compartment localized

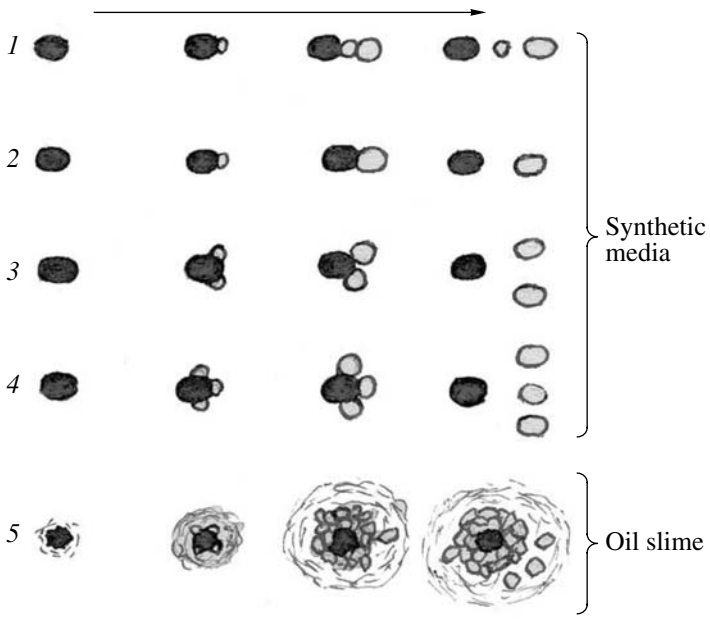

Fig. 9. Schematic picture of different versions (*1–4*) of the cell cycle of strains NF1 and NF3. Black circles represent preexisting individuals; light circles, the cells formed de novo by budding. *1*, formation of a large "final" cell through the stage of budding of an intermediate small cell; *2*, formation of a cell equal in volume to the initial (mother) one; *3*, concurrent formation of two buds on mother cell; *4*, concurrent formation of three buds simultaneously on mother cell; *5*, formation of spherical aggregates of budding nanocells in situ in oil slime (aggregates are enclosed in a thick capsule).

between the cytoplasmic and outer membranes. The periplasmic zone contains aggregates of electron-dense granules and large electron-dense spherical bodies (up to 20–30 nm in diameter), most often occurring in cocultures with cyanobacteria (Figs. 7, 8). Strains NF1 and NF3 are chemoorganotrophs, aerobes, catalase- and urease-positive, and capable of metabolizing a limited range of organic compounds: they utilize simple carbohydrates as a carbon source (see the Table); ammonium and nitrate nitrogen or amino acids as a nitrogen source; polymers (cellulose, pectin, agar) and proteins (gelatin, casein) are not decomposed. Growth factors are required: yeast extract or vitamins (pyridoxine, thiamine, riboflavin, nicotinic, *p*-aminobenzoic, and folic acids, biotin, and nicotinamide).

Interaction of strains NF1 and NF3 with CB. When incubated together with cultures of some cyanobacterial (CB) species, both UMB strains are able to lyse CB both in the light and in the dark. The CB cells with sheaths (S) proved to be the most sensitive to lysis: *Chlorogloeopsis fritschii* ATCC 27193 and *Chlorogloeopsis* sp. S. The strains *Anabaena variabilis* ATCC 29413, *Nostoc muscorum*, and *Spirulina* sp. 287 were less sensitive. The cyanobacterial biomass noticeably lost its green color already on day 3–4 of culture incubation; the yellow color predominated on day 4–7, and after 15–20 days the CB biomass was completely colorless. At the same time, a decrease and then disappearance of the red fluorescence of chlorophylls under ultraviolet excitation was observed by epifluorescence microscopy (Fig. 10). Fluorescence microscopy and electron microscopy showed that at the first stage of lysis the NF1 and NF3 cells were attached to the cyanobacterial cell surface. Then, UMB cells penetrated into the sheath: first into its loose surface capsular layer (Figs. 10, 11a) and then into the inner electron-dense layer with a lamellar structure (Fig. 11b). At the last (third) stage, UMB penetrate into CB cells, causing deep lysis of host cell cytoplasm (Fig. 11c). Since close contact between the fibrils of CB capsule and UMB capsule was observed at the primary contact of UMB and CB cells, carbohydrate–carbohydrate interactions obviously play an important role in cell–cell interactions (Fig. 11a). Cyanobacteria without developed capsules are less prone to be attacked by UMB (Fig. 10c). It should be mentioned that NF1 and NF3 grow well in co-culture with CB on an agarized medium for phototrophs [10] (without sources of nitrogen and organic carbon) and do not lose their viability for a long time (over 12 months). Yet, in the absence of CB, there is absolutely no growth of NF1 and NF3 on this medium. These data indicate that NF1 and NF3 exist under these conditions due to nutrients (including vitamins) synthesized by cyanobacteria. Thus, strains NF1 and NF3 can be defined as facultative parasites: ecto- and intracellular (the interaction of UMB with CB and other bacteria will be discussed in detail in a separate publication).

Systematic position of strains NF1 and NF3. According to the results of analysis of nearly complete nucleotide sequences of the 16S rRNA gene, a high degree of similarity (99.4%) exists between NF1 and NF3; they apparently belong to the same species. Due to the high level of similarity in the nucleotide sequence of the 16S rRNA gene (99.4%) and coincidence of the data on mol.% G+C content in chromosomal DNA of strains NF1 and NF3 and the type strains of the recently described species *Kaistia adipata* [20], the isolated bacterium was assigned to the above species; however, for the final conclusion it is necessary to obtain the data on their DNA homology. It is impossible to make a comparison on a number of phenotypic characteristics, because description of these (such as ultrastructural organization, cell cycle, and interrelations with CB) is missing in the original publication.

DISCUSSION

The data on UMB that we have found in oil slime (in situ) (Figs. 1a and 1b) show that free-living UMB actually occur and develop in slimes and soils; under normal natural conditions, they form extremely small coccoid cells (180–200 nm). The UMB isolated as pure culture is also able to form nanocells (200–300 nm) in the course of its development and cell cycle; many of them are invisible or poorly discerned by conventional light microscopy. The UMB observed in oil-slime and the UMB obtained as pure culture (strains NF1 and NF3) were highly similar in a number of significant and unique cytological characteristics. However, the indigenous UMB form (in situ) has a more marked tendency to form extremely small nanocells and a large capsule surrounding spherical cell aggregates. It should be noted that, apart from nanocells, strains NF1 and NF3 form large cells on synthetic nutrient media. The size of large cells and their nucleoids exceeds the size of nanocells by three to four times. Therefore, large cells might be considered as nondivided multicellular individuals. All the UMB described up to now are characterized by polymorphism, i.e., the presence of both very small and larger cells in the culture; (with the exception of *Pelagibacter ubique* [2]; however, the cytology of this bacterium is still poorly studied).

Bacteria of the genus *Kaistia* seem to be widespread in nature: we have found them in oil slime from Tatarstan (strain NF1) and in the near-root zone of an African plant *Pedilantus tithymaloides* (strain NF3). Strains of coccoid bacteria with cells analogous in size and ultrastructure to those of NF1 and NF3 have been isolated from the light chestnut soil of Prikaspiyskaya Lowland and silt from Lake Baikal (strain NF2). Similar bacterial nucleotide sequences of the 16S rRNA gene presented in GenBank have been revealed in Belgium, USA (accession numbers AY 040361, AY 039817, and DQ 337585), and Korea (type strain of the species *K. adipata*). A distinctive feature of the isolated UMB is its ability to interact with living cyanobacterial (CB) cells, with adsorption of UMB cells on the surface of CB cells, penetration of UMB into polysaccharide

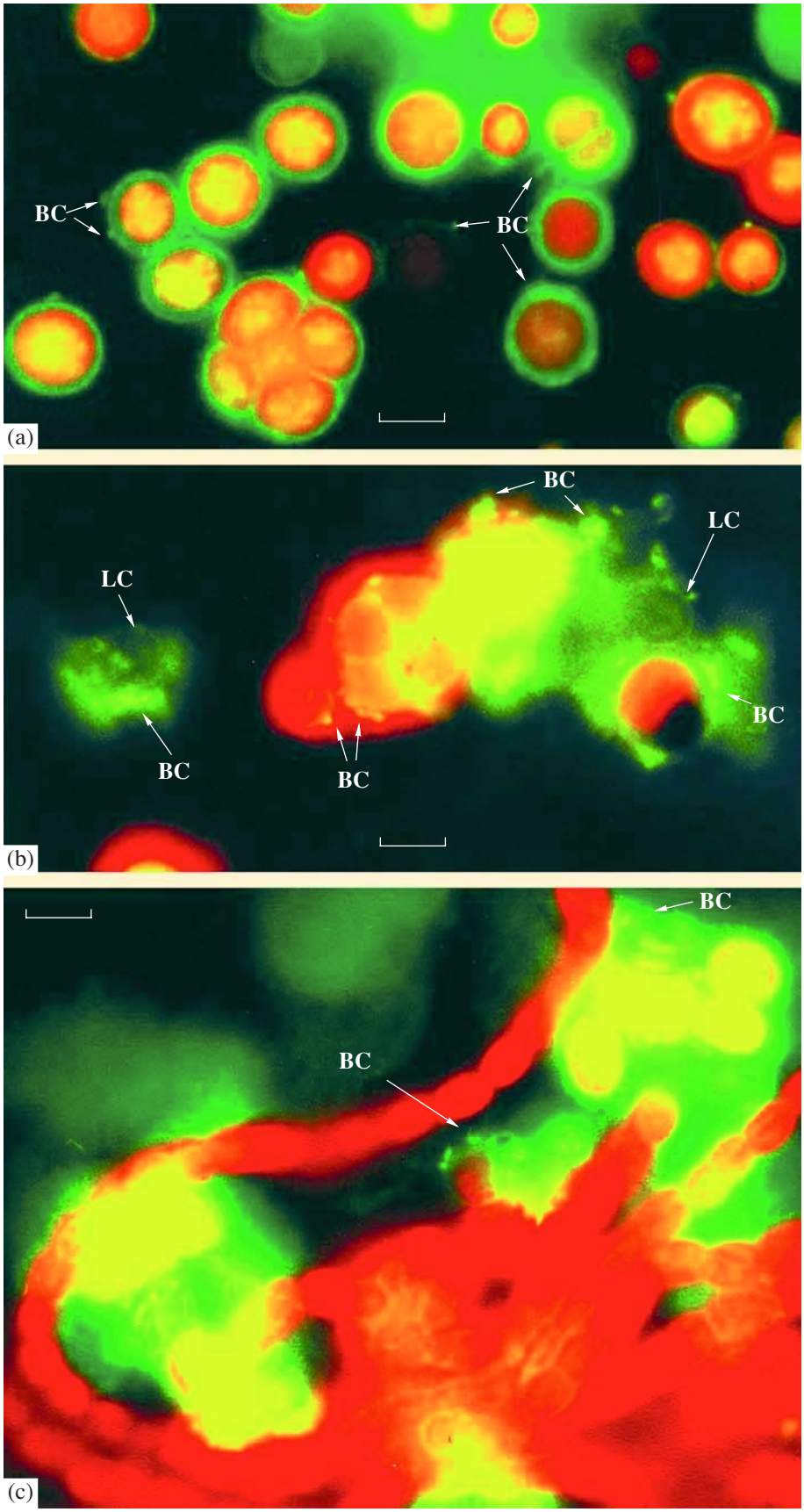

Fig. 10. Lysis of cyanobacteria by NF1. The effect on: (a), (b) *Chlorogloeopsis fritschii* ATCC 27193; (c) *Anabaena variabilis* ATCC 29413. Figure 10c shows that mainly heterocysts are attacked. Designations: BC, bacterial cells of strain NF1 (arrows); LC, lyzed cyanobacterial cells. Staining with DAPI. Scale bar is 5 μm.

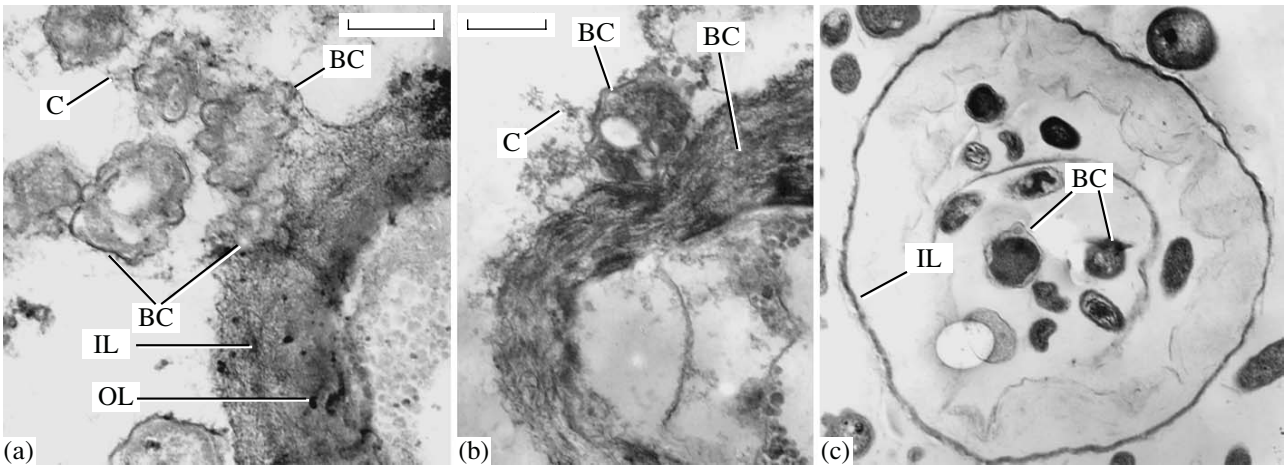

Fig. 11. Ultrathin sections. The lysis of *Chlorogloeopsis fritschii* ATCC 27193 cells is shown. The cells are strained with ruthenium red. Scale bar is 3 μm in Figs. a and b and 1 μm in Fig. c. Designations: BC, bacterial cells of strain NF1; EL, external layers of the sheath; IL, internal layer of the sheath of cyanobacterial cells. Scale bar is 0.3 μm.

sheaths, and penetration into CB periplasm. Thus, the isolated UMB may be described as a facultative parasite. Although some bacteria (bdellovibrios [21] and cytophagas [22]) are known to induce extracellular lysis of cyanobacteria, bacterial parasites which can penetrate into the envelopes and cytoplasm of cyanobacterial cells have not been isolated and described as yet. Our findings demonstrate that UMB include spe-

Characteristics of strains NF1 and NF3

Characteristics	Strain NF1	Strain NF3
Cell shape and size	Coccoid, rarely rod-shaped, 0.3 and 0.4–0.8 μm in diameter	Coccoid, rarely rod-shaped, 0.3 and 0.4–0.8 μm in diameter
Type of envelope	gram-negative	gram-negative
Reproduction	Budding	Budding
Attitude to oxygen	Aerobe	Aerobe
Cytochrome composition	*a*, *b*, *c*	*a*, *b*, *c*
Catalase	+	+
Fatty acid composition of membrane lipids	$C_{14–18}$ – fatty acids, $C_{19:0}$-cyclo predominates	not detected
Presence of pili and flagella	–	–
Utilization as sole carbon source	Simple sugars and some organic and amino acids*	The same as for strain NF1
Vitamin requirements	+	+
Resistance to streptomycin (50 μ g/ml)	+	+
Sensitivity to antibiotics**	+	+
Presence of plasmids	–	–
Parasitism on cyanobacteria	+	+

Notes: * D-glucose, D-fructose, D-xylose, D-arabinose, L-rhamnose, D-maltose, D-sucrose, D-lactose, mannitol, glycerol, inositol, dulcitol, pyruvic and succinic acids, casamino acids.

** Chloramphenicol (20 μg/ml); penicillin (50 μg/ml); kanamycin (25 μg/ml); gentamycin (25 μg/ml); rifampicin (50 μg/ml); novobiocin (50 μg/ml); claforan (50 μg/ml); tetracycline (10 μg/ml); ampicillin (500 μg/ml).

cies with new, previously unknown physiological properties and ultrastructures.

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