FINE STRUCTURE OF FOSSILIZED BACTERIA IN VOLYN KERITE

V. M. GORLENKO[1], S. I. ZHMUR[2], V. I. DUDA[3], N. E. SUZINA[3], G. A. OSIPOV[4] and V. V. DMITRIEV[3*]

[1] *Institute of Microbiology of Russian Academy of Sciences, Moscow, Russia*
[2] *Institute of the Lithosphere of Marginal Seas, Moscow, Russia*
[3] *G.K. Skryabin Institute of Biochemistry and Physiology of Microorganisms of Russian Academy of Sciences, Pushchino, Russia*
[4] *Filatov Pediatric Hospital, Moscow, Russia*
([*] To whom correspondence should be addressed: IBPM RAS, Prospekt Nauki, 5, Pushchino, 142292 Russia, e-mail: dmitriev@ibpm.serpukhov.su)

(Received 8 December, 1999; accepted in revised form 14 July, 2000)

Abstract. Ultrathin sectioning and cryofracture of fibrous kerite, sampled from 1.8–1.75 billion year old Volyn sediments (Ukraine), revealed in bacteria-like bodies the presence of structures similar to sheath, cell wall, periplasm, cytoplasm, septum, membranes, intramembrane particles, poly-β-hydroxybutyrate inclusions. On the strength of these data and also the fatty acid profiles of these microfossils, we concluded that fibrous kerites are biogenic formations, namely fossilized bacterial mats.

Keywords: ancient cyanobacterial mats, biogenic origin, fatty acids, kerite, microfossils, ultrastructure

1. Introduction

The biological origin of some fossilized materials is still disputable despite some circumstantial evidence to the contrary. One of the reasons is the difficulty of their preparation for high-resolution electron microscopy, which can reveal the ultrastructures inherent of biological objects. Prokaryote microfossils found in sedimentary rocks are most often diverse lithified cells (Shimizu *et al*., 1978; Mojzsis *et al*., 1996; Hoover *et al*., 1998; Herman, 1990; Horodyski and Knauth, 1994) usually characterized at the light microscopy level (5–35 μm sections). Scanning electron microscopy, well-accepted by paleontologists, has added some features to the morphological image of microfossils while their ultrastructure still remains virtually neglected in spite of the fact that ultrastructural data could be decisive factors in the elucidation of the kind and origin of microfossils as well as of their possible occurrence in meteorites and other space objects.

A unique example in this respect is the fibrous kerite of Volyn. The origin of its filamentous and rod-like constituents remains obscure hitherto. Fibrous kerite was discovered in the course of 80 m deep mining of topaz-morion pegmatic bodies in the Korostinsk granite pluton in the Volyn region of the Ukraine (Ginzburg *et al*.,

Origins of Life and Evolution of the Biosphere **30:** 567–577, 2000.

1987; Luk'yanova *et al*., 1992). Middle Proterozoic pegmatites are considered to be syngenetic to surrounding granites 1.8–1.75 billion years of age. The kerite was observed as the mass of interwoven fibers (reminiscent of black felt or slightly pressed fiber) that filled the central void inside the pegmatite body and spaces in-between the quartz, feldspar, and topaz crystal fragments. Sometimes kerite assembalges might be as heavy as 3 kg. By its chemical characterstics, fibrous kerite corresponds to higher oxidized kerites. The simplest formula of Volyn kerite (corresponding to hard bitumoids) was determined as $C_{491}H_{386}O_{87}$(S)N (Ginzburg *et al*., 1987). The nitrogene concentration was unusually high (8–9%); sulfur made up 2–3% of the total organic matter. The diffraction and spectroscopic examinations disclosed a complex structure of fibers; it was suggested that fibrous kerite has an ordered polymeric structure and, besides hydrocarbon polymers, contains hydrocarbon in a prographite phase. The filamentous and rod-like bodies that consitiute this type kerite were attributed by its investigators (Ginsburg *et al*., 1987; Luk'yanova *et al*., 1992; Yushkin, 1996, 1998) to structures of the abiogenic origin.

Kerite represents a mass of interwoven hard bitumoid filaments liable for high-resolution electron-microscopic examination. Our interest to the origin of fibrous kerite impelled us to investigate its ultrastructure and fatty acids composition. The peculiar morphology of fibrous kerite (the presence of organelle-like structures similar to sheath, cell wall, periplasm, cytoplasm, septa, membranes, intramembrane particles, poly-β-hydroxybutyrate inclusions), the size and assemblage of its filaments in a network reminiscent of felt (all these typical of cyanobacterial mats), as well as the availability of fatty acids peculiar of bacteria, point out to its biogenic nature.

2. Materials and Methods

Samples: Kerite (sample BK N564557 from the Volyn) was obtained from V.I. Vernadsky Mineralogical Museum.

Electron microscopic examinations:

(1) *Ultrathin sections*. Kerite was fixed with 1.5% glutaraldehyde solution in 0.05 M cacodylate buffer (pH 7.2) at 4 °C for 1 hr; washed thrice with the same buffer; and fixed again with 1% OsO_4 solution in 0.05 M cacodylate buffer (pH 7.2) for 3 hr, at 20 °C. After dehydration, material was embedded in Spurr epoxy resin. Ultrathin sections, made on the LKB Ultramicrotome III, were stained with 3% uranyl acetate solution in 70% alcohol for 30 min, and lead citrate at 20 °C for the next 4–5 min according to (Reynolds, 1963).

(2) *Freeze-fracture*. Prior to cryofixation, the specimen was placed in a drop of bidistilled water at 20 °C. The material was then frozen in cooled propane (–196 °C). Cryofracture was done in a JEE-4X vacuum device, as described in (Fikhte *et al*., 1973), under vacuum of 3×10^{-4} Pa at the specimen temperature

of –100 °C. Replicas were obtained by coating the fracture surface (under vacuum) with platinum-carbon mixture and pure carbon at the angles of 30 and 90°, respectively.

(3) *Fatty acid composition*. Kerite samples (0.07 g) were subjected to acid methanolysis in 0.5 mL dried HCl in methanol by heating to 80 °C for 3 hr. The resulting fatty acid methyl esters were extracted with hexane. The hexane fractions were dried, and the dry residue was sylylated in 20 μL N,O-bistrimethylsilyltrifluoroacetamide by heating at 80 °C for 15 min. Measurements were performed on a Shimadzu GC-MS QP-2000 spectrometer equipped with a cross-linked methyl silicone capillary column (Ultra-1). The oven temperature was maintained at 120 °C for 2 min and then gradually increased to 320 °C at a rate of 5 °C per min (Osipov and Turova, 1997).

3. Results and Discussion

The kerite samples represented interwoven filaments of various diameter (0.3 to 35 μm, Figure 1a), typical of cyanobacterial mats. Some of the filaments could branch and intergrow forming swelling at joints, thick filaments would split to clusters of the thinner ones. From their tendency to orthogonal fragmentation, we assumed the availability of cross septa. The filaments of 3 to 8 μm in diameter represented chains of cell-like structures (Figures 1b–g). Their inner appearance resembled the cytoplasm of living cells embedded in a multilayer envelope 100–200 nm thick. The envelope consisted of a sheath (130 nm), a cell wall (60–80 nm) and a membranous layer (Figures 1b–g). The first two were easily detachable from the cell. In between the cell wall and the 'cytoplasm', there was an easily distinguishable periplasm taking the shape of thin (10 nm) and sometimes abruptly expanding (to 50–60 nm) electron transparent layer (Figures 1d and g). Similar layered formations covered also the empty filaments, and probably were the envelopes of filamentous microorganisms related to cyanobacteria and filamentous green bacteria. The filaments were composed of cells separated by septa being in fact double cell wall layers 120–200 nm thick. The electron transparent space in between the layers was typical of cell septa (Figure 1f). The membranes looked as a thin electron-dense layer on the periphery of a cytoplasmic cylinder, however its triplet structure was not revealed. The cytoplasm-like compartment of filaments was usually dense, fine-grained or folded. In some cells, it was partially disturbed and represented adjacent accumulations of loose coarse-grained and dense fine-grained matter. These patterns are typical of lysing cells. The 'cytoplasm' sometimes contained inclusions whose structure and density resembled polyphosphates granules (dark bodies) found in prokaryotic cells (Figures 1c and f). However, structures comparable with intracytoplasmic membranes, and in particular with the photosynthetic apparatus, were almost absent on the sections.

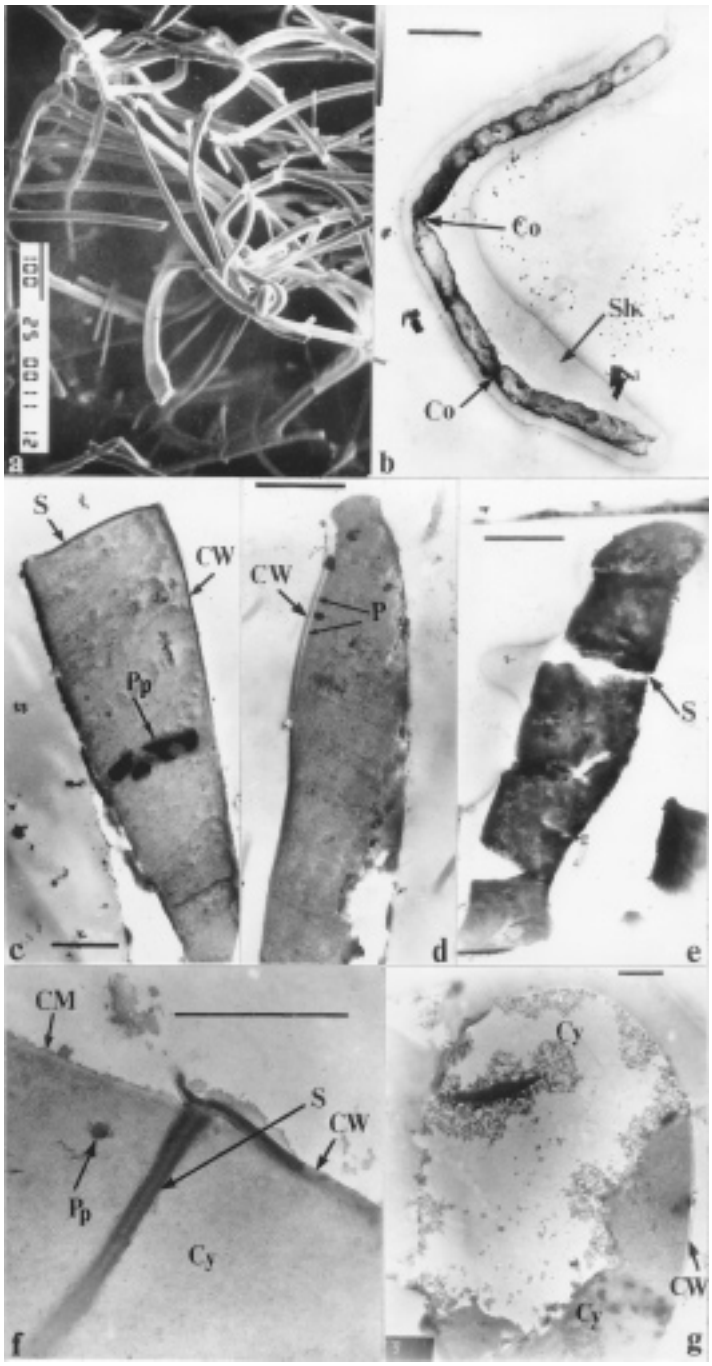

Figure 1. Electron microscopy of filamentous bacteria-like structures in fibrous kerite. (a) scanning electron microscopic image; (b–f) ultrathin sections CW – cell wall, CM – cytoplasmic membrane, Co – constriction, Cy – cytoplasm, S – septum, Sh – sheath, P – periplasmic space, Pp – polyphosphate granules. Scale: 1 μm.

Not very numerous were thinner filaments (0.3–0.5 μm in diameter) representing encapsulated trichomes of cells divided by septa and constrictions (Figure 1c). Morphologically they were similar to filamentous green bacteria of the *Chloroflexus* group, accompanying cyanobacteria in marine and hydrothermal mats.

The cryofracture of fibrous kerite yielded additional evidences of the cellular structure of filaments. The lateral fractures revealed cross septa and cell walls (see the electron dense layers of varying thickness in Figure 2a). The inner was homogenous matter which became fine-grained in the course of fossilization; it is usually not observed in vegetative bacterial cells whose cytoplasm is more coarse-grained due to polyribosomes.

We also observed inclusions (0.1–0.5 μm), structurally almost identical to poly-β-hydroxybutyrate granules found in contemporary bacteria (Remsen, 1966; Dunlop and Robards, 1973; Reusch *et al*., 1987). On metal-shadowed replicas, they looked like coned granules whose tops produced large tongue- or torch-like light shadows. It was also seen clearly, that the granules were enveloped by a bazal 'membrane' (wall) whose surface looked like a smooth, structure-less layer (Figure 2b).

The cryofracture of the 'cytoplasm' envelope revealed its structural similarity to the cytoplasmic membrane of existing bacteria: smooth areas of 'pure' lipids alternated with regions of tightly packed intramembrane particles of 8–12 nm (Figures 2a–c). In modern microorganisms, similar particles are considered integral membrane proteins (Murray, 1978; Mayer, 1999). The surface of some filaments was covered with a fragmented layer consisting of chaotically arranged aggregates of smooth polygonal leaflets (Figure 2c), which probably were remnants of the degraded outer membrane of Gram-negative bacteria.

The electron microscopic examinations of fossils revealed a number of ultrastructures in bacteria-like forms that had unexpectedly good resolution and were similar to analogous structures of modern bacteria. The most showing in this respect were such structures as cell walls and poly-β-hydroxybutyrate granules. However, in the course of fossilization, these 'cells' underwent profound changes: they have no nucleoids and intracytoplasmic membrane structures; some fossils partially or completely lost cell walls (Figure 1f) and sheats; in some bacteria-like forms, a cytoplasmic component has a fine-grained structure (Figure 1f); in the others, the cytoplasm was completely or partially degraded; the cytoplasmic membrane is not revealed on the sections as a triplet structure; the structure, analogous to the outer membrane of the cell wall is either absent or has, evidently, undergone profound changes, it is present in the form of fragments, leaflets of membrane-like structures (Figure 2c) (such structures are usually found in gram-negative bacteria in autolysing suspensions).

One should take into account profound post-mortal changes in fossils, in particular at the ultrastructural level (Oehler, 1977; Ferris *et al*., 1986). Besides, additional artefacts can be the results of specimen pretreatment. Nevertheless, the data on the fossil ultrastructure can be of value in studies of the biological nature of

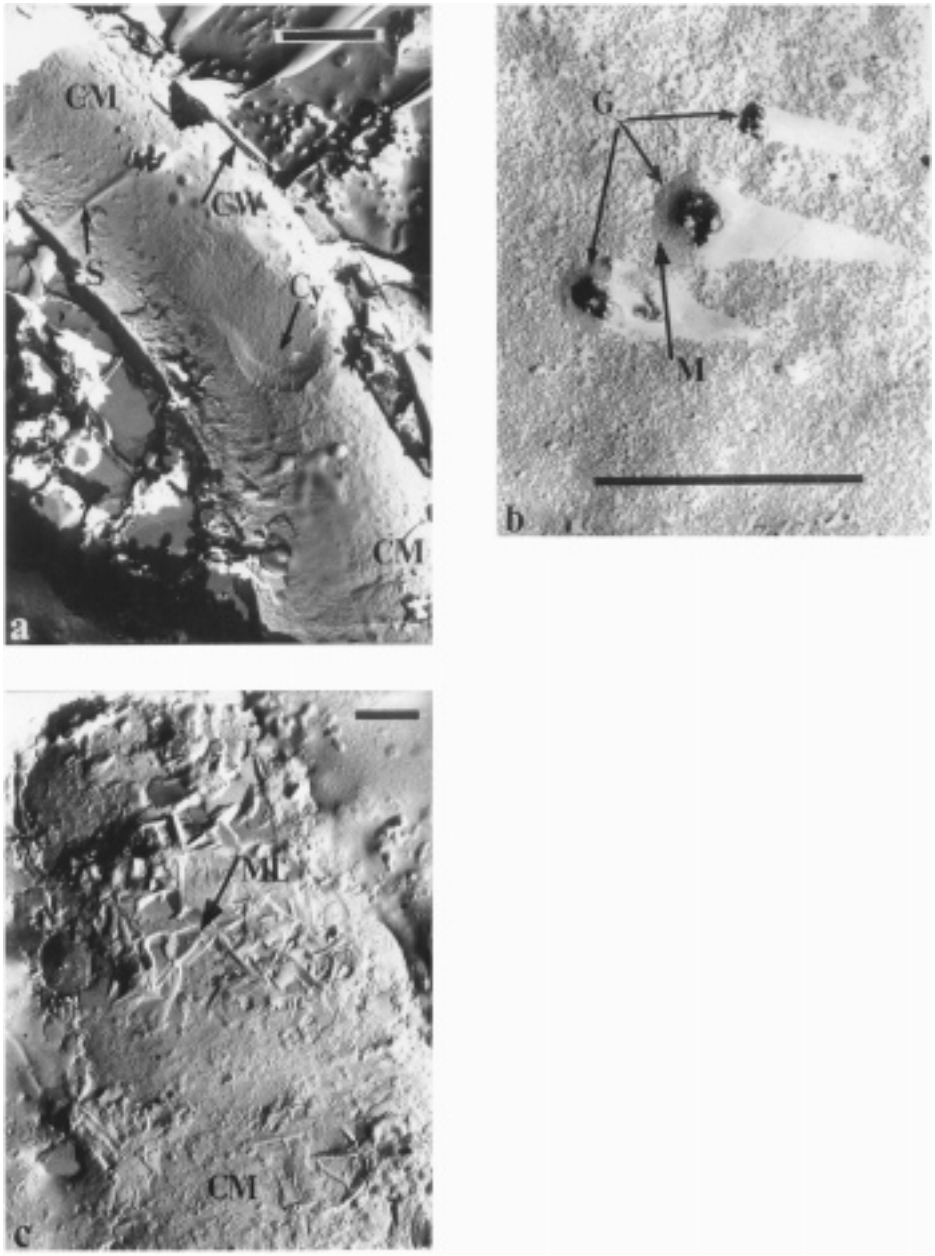

Figure 2. Electron microscopy of cryofracture replicas of filamentous bacteria-like structures in fibrous kerite. Designations are as in Figure 1. Additional: G – poly-β-hydroxybutyrate granules; M – 'membrane' of poly-β-hydroxybutyrate granules; ML – membrane leaflets. Scale: 1 μm.

some organic structures or taxonomic affiliation of some fossils.

In scales of geological time, the life-time of cell structures of ancient microbial forms has yet been scantily studied. The essential cell structures can be preserved in the almost native state for 25–40 million years; this fact is confirmed by the isolation of living bacteria from amber (Cano and Borucki, 1995). We found viable yeasts in 3 million years old Siberian permafrost (Dmitriev *et al.*, 1998). It is supposed that in dead fossilized microorganisms, cell structures can be preserved for much longer time. The possibility of the ultrastructural analysis of fossilized organisms has been reported in recent publications (Poinar and Hess, 1982; Li *et al.*, 1998), among those soft-bodied fossils from Silurian volcaniclastic deposits (Briggs *et al.*, 1996).

The present article describes a number of ultrastructures in bacteriomorphic fossils of kerite associated with Paleoproterozoic pegmatite in the Ukraine. Their quite good resolution is evidently connected with a peculiar type of mummification of bacterial cells in fibrous kerite: carbonization and impregnation of the biological material with hydrocarbons (specifically bitumoids) present in kerite. There are no indications on the silicification, calcification, phosphorization, limonitization or impregnation of the studied fossils with other minerals substances. The analysis of ultrathin sections of fossils was supplemented with studies of cryofracture replicas. The freeze-etching method is valuable for the absence of preparative artefacts which are usual in ultrathin sectioning.

The main components of the kerite lipid fraction methanolysate were saturated straight-chain or branched fatty acids and hydroxy acids (Table I). It is common knowledge that C_{12--19} fatty acids are indicative of the presence of bacteria. Gram-negative bacteria (cyanobacteria) are evidenced by 3-hydroxyacids, the components of their cell wall outer membrane lipopolysaccharide (Lechevalier, 1977). The fatty acid profile of kerite is similar to that one of modern microbial communities surviving in unfavorable environment (Figure 3).

The comparison of the fatty acid composition of the Volyn kerites, cyanobacterial mats form the hyper saline Solar Lake (Sinai), and microbial mats from Kamchatka hot springs revealed a certain similarity of their profiles (Figure 3). This fact, and also the size and morphological similarity of some filaments and felt-like structure of the entire association, suggest that kerites of Volyn are evidently fossilized remnants of a benthonic cyanobacterial association. Their occurrence in pegmatic bodies in granite pluton points out to volcanic and hydrothermal activities taking place in the past. Most probable, the maternal cyanobacterial mat of Volyn kerite developed first in the surface thermal spring.

Fatty acids were not abundant in Volyn kerites. The absence of unsaturated fatty acids excludes the appearance of these compounds due to contamination. It was shown earlier in the organic matter of the buried mat of the Solar lake, the quantity of unsaturated fatty acids decreased with depth until their complete absence (with the simultaneous appearance of protokerogen in rocks). The absence of unsaturated

TABLE I
Fatty acids of kerite

No.	Short name[a]	Quantity (μg g^{-1})	Chemical name
1	12:0	3.24	Lauric
2	13:0	0.43	Tridecanoic
3	i14	0.15	*iso*-myristic
4	14:0	6.93	Myristic
5	i15	0.38	*iso*-pentadecanoic
6	a15	0.63	*anteiso*-pentadecanoic
7	15:0	3.52	Pentadecanoic
8	16:1Δ9	3.16	9,10-hexadecanoic
9	i16:0	0.26	*iso*-palmitic
10	16:0	29.3	Palmitic
11	i17:0	0.25	*iso*-heptadecanoic
12	a17:0	0.61	*anteiso*-pentadecanoic
13	17:0	2.2	Heptadecanoic
14	18:1Δ9	5.3	9,10-octadecanoic
15	18:0	16.7	Stearic
16	19:0	0.02	Nonadecanoic
17	10Me16	1.0	10-methyl-hexadecanoic
18	10Me18	0.01	10-methyl-octadecanoic
Hydroxy acids			
19	h12[b]	0.01	Hydroxy-lauric
20	h14	0.01	Hydroxy-myristic
21	h16	0.09	Hydroxy-palmitic
22	10h18	0.07	10-hydroxy-stearic
23	h18	0.04	Hydroxy-stearic
24	2h12	0.02	2-hydroxy-lauric
25	Σ	74.6	

[a] Marking: (16.1–16) is the number of carbon atoms, the figure after colon denotes the number of double bonds; h is hydroxy-acid; a, i = indicate methyl-branching, e.g. 2h12 means 2-hydroxy-lauric acid.
[b] = 3-hydroxy-acid – the position of hydroxyl, not indicated.

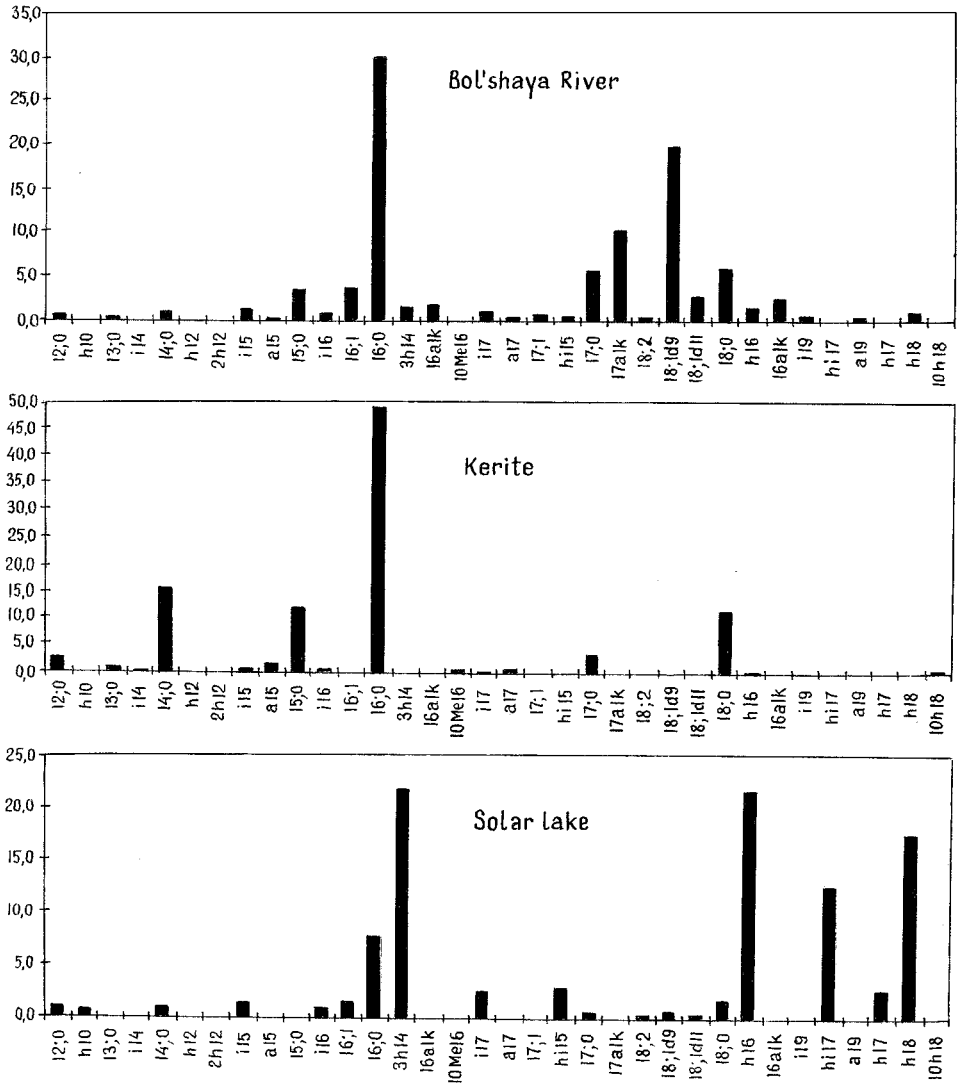

Figure 3. Fatty acid profiles (% of total) of kerite compared with cyanobacterial mats of Bol'shaya River (our data) and Solar Lake (Frederickson, 1989).

fatty acids in Volyn kerites can be explained by the earlier happened processes of diagenesis.

Lipids in organic matter of ancient sedimentary rocks, schist, and oil are assumed to be fossilized remnants of molecules of prokaryotic organisms (Michaelis and Albrecht, 1979; De Rosa *et al.*, 1982; McCafrey *et al.*, 1989; Peters and Moldowan, 1993; Brocks *et al.*, 1999). Other organic macromolecules of the biological origin can also be preserved in sedimentary rocks for quite a long time; e.g. chitin was found in 25 million year old fossils (Stankiewicz *et al.*, 1997).

On the strength of the data on the ultrastructure of filaments and rod-like forms and the fatty acid composition of fibrous kerite, we assume that this microfossil is an ancient cyanobacterial mat probably of the hydrothermal origin.

Acknowledgements

The work was supported by Grants 99-04-49145; 99-04-48707; and 98-0554-765, 99-04-49144 of the Russian Foundation for Fundamental Research. We thank Professor L. V. Kalakoutsky and V. A. Dmitrieva for valuable remarks and L. L. Mitjushina for technical assistance.

References

Briggs, D. E. G., Siveter, D. J. and Siveter, D. J.: 1996, Soft-Bodied Fossils from a Silurian Volcaniclastic Deposit, *Nature* **382**, 248–250.

Brocks, J. J., Logan, G. A., Buick, R. and Summons, R. E.: 1999, Archean Molecular Fossils and the Early Rise of Eukaryotes, *Science* **285**, 1033–1036.

Cano, R. J. and Borucki, M. K.: 1995, Revival and Identification of Bacterial Spores in 25–40 Million-Year-Old Dominican Amber, *Science* **268**, 1060–1064.

De Rosa, M., Gambacorta, A., Nicolaus, B., Ross, H.-N.-M., Grant, W. D. and Bu'Lock, J. D.: 1982, An Asymmetric Archaebacterial Diether Lipid from Alkalifilic Halophiles, *J. Gen. Microbiol.* **128**, 343–348.

Dmitriev, V. V., Gilichinskii, D. A., Faizutdinova, R. N., Shershunov, I. N., Golubev, V. I. and Duda, V. I.: 1997, Detection of Viable Yeast in 3-Millio-Year-Old Permafrost Soil of Siberia, *Microbiology* **66**, 655–660 (English translation).

Dunlop, W. F. and Robards, A. W.: 1973, Ultrastructural Study of Poly-β-Hydroxybutyrate Granules from Bacillus cereus, *J. Bacteriol.* **114**, 1271.

Ferris, F. G., Beveridge, T. J. and Fyfe, W. S.: 1986, Iron-Silica Crystallite Nucleation by Bacteria in a Geothermal Sediment, *Nature* **320**, 609–611.

Fikhte, B. A., Zaichkin, E. I. and Ratner, E. N.: 1973, New Methods for Physical Treatment of Biological Objects for Electron Microscopy, Nauka, Moscow, 1–148 (in Russian).

Fredrickson, H. L., Rijpstra, W. I. C., Tas, A. C., Van der Greef, J., La Vos, G. F. and De Leeuw, J. W.: 1989, Chemical Characterization of Bentic Microbial Assemblages, in Y. Cohen and E. Rosenberg (eds), *Microbial Mats. Physiological Ecology of Bentic Microbial Communities*, Chapter 39, pp. 455–468.

Ginsburg, A. I., Bulgacov, V. S., Vasilishin, I. S., Lukyanova, V. T., Solntseva, L. S., Urmanova, A. M. and Uspensky, V. A.: 1987, *Kerite from Volyn' Pegmatites*, Doklady Akademii Nauk SSSR, 292, 188–191 (in Russian).

Herman, T. N.: 1990, *Organic World Billion Year Ago*, Nauka, Leningrad, 1–50.

Hoover, R. B., Rozanov, A. Yu., Zhmur, S. I. and Gorlenko, V. M.: 1998, Further Evidence of Microfossils in Carbonaceous Chondrites, *Proc. SPIE* **3441**, 203–216.

Horodyski, R. J. and Knauth, L. P.: 1994, Life on Land in the Precambrian, *Science* **263**, 494–498.

Lechevalier, M. P.: 1977, Lipids in Bacterial Taxonomy – A Taxonomist's View, *Crit. Rev. Microbiol.* **5**, 109–210.

Li, Ch.-W., Chen, J.-Y. and Hua, T.-E.: 1998, Precambrian Sponges with Cellular Structures, *Science* **279**, 879–882.

Lukyanova, V. T., Lobozova, R. V. and Popov, V. T.: 1992, *Fibrous Kerite from Volyn' Pegmatites*, Izv. Akad. Nauk SSSR, Ser. Arkheologicheskaya **5**, 102–118 (in Russian).

McCaffrey, M. A., Farrington, J. W. and Repeta, D. J.: 1989, Geochemical Implications of the Lipid Composition of Thioploca spp. from the Peru Upwelling Region – 15°S, *Org. Geochem.* **14**, 61–68.

Mayer, F.: 1999, Cellular and Subcellular Organization of Prokaryotes, in *Biology of the Prokaryotes*, Blackwell-Science, Thieme, Stuttgart, New York, pp. 20–46.

Michaelis, W. and Albrecht, P.: 1979, Molecular Fossils of Archaebacteria in Kerogen, *Naturwissenschaften* **66**, 420–422.

Mojzsis, S. J., Arrhenius, G., McKeegan, K. D., Harrison, T. M., Nutman, A. P. and Friend, C. R. L.: 1996, Evidence for Life on Earth before 3800 Million Years Ago, *Nature* **384**, 55–59.

Murray, R. G. E.: 1978, Form and Function – I. Bacteria, in Norris J. R. and Richmond M. H. (eds), *Essays in Microbiology*, J. Wiley & Sons, Chichester, pp. 2/1–2/31.

Oehler, D. Z.: 1977, Pyrenoid-Like Structures in Late Precambrian Algae from the Bitter Springs Formation of Australia, *J. Paleontol.* **51**, 885–901.

Osipov, G. A. and Turova, S.: 1997, Studying Species Composition of Microbial Communities with the Use of Gas Chromatography – Mass Spectrometry: Microbial Community of Kaoline, *FEMS Microbiology Reviews* **20**, 437–446.

Peters, K. E. and Moldowan, J. M.: 1993, *The Biomarker Guide* (Interpreting molecular fossils in petroleum and ancient sediments), A Simon and Shuster Co., Englewood Cliffs, New Jersey.

Poinar, G. O. and Hess, R.: 1982, Ultrastructure of 40-Million-Year-Old Insect Tissue, *Science* **215**, 1241–1242.

Remsen, C. C.: 1966, The Fine Structure of Frozen-Etched Bacillus cereus spores, *Arch. Microbiol.* **54**, 266–275.

Reusch, R., Hiske, T., Sadoff, H., Harris, R. and Beveridge, T.: 1987, Cellular Incorporation of Poly-β-Hydroxybutyrate into Plasma Membranes of Escherichia coli and Azotobacter vinelandii Alters Native Membrane Structure, *Can. J. Microbiol.* **33**, 435–444.

Reynolds, E. S.: 1963, The Use of Lead Citrate at High pH as an Electron-Opaque Stain in Electron Microscopy, *J. Cell Biol.* **17**, 208–213.

Shimizu, A., Imahori, K. and Ykasa, S.: 1978, Examination of the Gunflint Microfossils by Scanning Electron Microscopy, *Origins of Life* **8**, 527–532.

Stankewicz, B. A., Briggs, D. E. G., Evershed, R. P., Flannery, M. B. and Wuttke, M.: 1997, Preservation of Chitin in 25-Million-Year-Old Fossils, *Science* **276**, 1541–1543.

Yushkin, N. P.: 1996, Natural Polymer Crystal of Hydrocarbon as Model of Prebiological Organisms, *J. of Crystal Growth* **167**, 237–247.

Yushkin, N. P.: 1998, Hydrocarbon Crystals as Protoorganisms and Biological Systems Predecessors, *Proc. SPIE* **3441**, 234–246.