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EXPERIMENTAL ARTICLES

Taxonomic Characterization of the Microorganisms Associated with the Cultivable Diatom *Synedra acus* from Lake Baikal

Yu. R. Zakharova[1], R. V. Adel'shin, V. V. Parfenova, Ye. D. Bedoshvili, and Ye. V. Likhoshway

Limnological Institute, Siberian Branch, Russian Academy of Sciences, ul. Ulan-Batorskaya 3, Irkutsk, 664033, Russia

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Abstract—Determination of the taxonomic position of microorganisms associated with diatoms was carried out using microscopic, microbiological, and phylogenetic methods. Examination of the cultures of the freshwater diatom *Synedra acus* grown in Lake Baikal water by epifluorescence and electron microscopy revealed that bacterial cells colonized the mucilage surrounding the cells of living microalgae and their cell surfaces and penetrate into the frustules of dead diatoms. A total of 13 strains of heterotrophic bacteria were isolated in pure cultures and described. Based on the results of analysis of their morphological, physiological, and biochemical properties, as well as on data obtained by 16S rRNA gene analysis, the following strains were identified to the species level: *Sphingomonas* sp., *Variovorax paradoxus, Pseudomonas fluorescens, Microbacterium trichothecenolyticum, Rhodococcus* sp., *Caulobacter vibrioides*, and *Brevundimonas vesicularis.*

Key words: microorganisms, diatoms, Lake Baikal, 16S rRNA, epifluorescence, scanning, and transmission electron microscopy

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Bacteria and diatoms coexist in aquatic ecosystems and interact in different ways. The majority of published data are results obtained in studies of marine ecosystems. The dependence of diatoms on bacterial metabolites (e.g., vitamins) has been demonstrated [1, 2]. Bacteria, in turn, depend on the metabolites secreted by diatoms [3–5]. Cultivation experiments revealed that different microorganisms were associated even with the diatoms isolated from the same source. Moreover, the succession of bacterial morphotypes of epiphytic bacteria attached to the surfaces of diatom cell during transition from the active growth phase of microalgae to the stationary phase has been described [4]. Determination of the role of bacteria in the destruction of diatoms during their precipitation and diagenesis is one of the aspects studied over the last decade that has aroused considerable scientific interest [6].

The issues concerning the role of microorganisms in the silicon cycle in the World Ocean have been studied and discussed in a number of works. It was demonstrated that the rate of in situ dissolution of silica frustules of marine diatoms increases significantly due to the activity of marine bacteria [7–9] belonging to specific phylotypes, namely, *Alphaproteobacteria*, *Betaproteobacteria, Gammaproteobacteria*, the *Cytophaga–Flavobacterium–Bacteroides* (*CFB*) group, *Actinobacteria*, and *Bacillus* [10–12].

There are considerably fewer works dealing with the interactions between microorganisms and freshwater diatoms. The main bacterial satellites (*Proteobacteria* and *Bacteroidetes*) inhabiting diatomaceous mats and diatom cultures from freshwater ecosystems were revealed [13]. It is also well known that *Pseudomonas putida* (class *Gammaproteobacteria*) exhibits antagonistic activity against the freshwater diatom *Stephanodiscus hantzschii* [14].

The freshwater diatom *Synedra acus* subsp. *radians* (Kütz.) Skabitsch. is one of the subjects of the investigations aimed at studying the mechanisms involved in the transport of silicic acid into the cell, as well as in silica biomineralization [15–17], the processes that have attracted a great deal of attention of biotechnologists. Recently, a method for large-scale cultivation of the diatom *Synedra acus* was developed, which made it possible to obtain large amounts of biomass (tens of grams per week in a 100-l photobioreactor). The use of biomass is aimed at obtaining biogenic silica, DNA, proteins, and lipids, including eicosapentaenoic acid [18]. In the course of diatom cultivation, the presence of the accompanying microflora was detected; in some cases, the growth of algae slowed down and they died.

The aim of the present work was to determine the taxonomic composition, as well as the physiological and biochemical properties of the bacteria associated with the Baikal freshwater diatom *Synedra acus* during its cultivation.

MATERIALS AND METHODS

Isolation and cultivation. A culture of the diatom *Synedra acus* subsp. *radians* was isolated from a natural population of the Listvenichnyi Bay of Lake Baikal.

[1] Corresponding author; e-mail: zakharova@lin.irk.ru

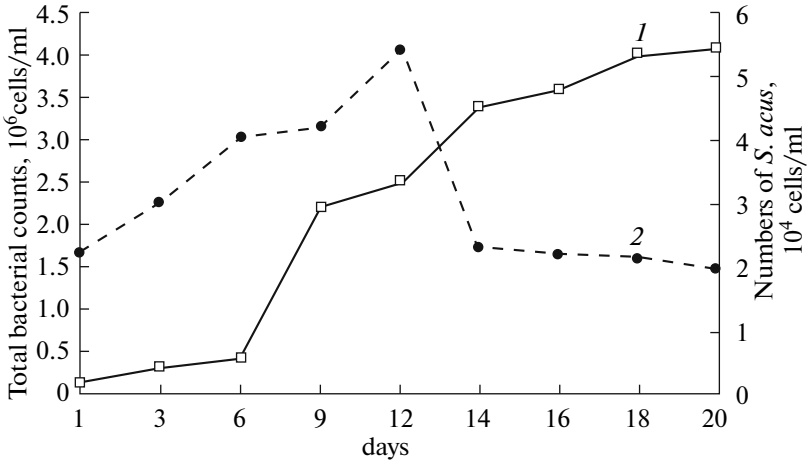

Fig. 1. Growth dynamics of the diatom *Synedra acus* and accompanying bacteria during cultivation: total bacterial number (*1*) and *S. acus* number (*2*).

The culture was grown in a 100-l photobioreactor as described earlier [18] on DM1 medium containing the following, mg/l: $Ca(NO_3)_2 \cdot 4H_2O$, 20; KH_2PO_4, 12.4; $MgSO_4 \cdot 7H_2O$, 25; $NaHCO_3$, 16; Na_2EDTA, 2.25; H_3BO_3, 2.48; $MnCl_2 \cdot 4H_2O$, 1.39; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 1.0; cyanocobalamin (vitamin B_{12}), 0.04; thiamine hydrochloride (vitamin B_1), 0.04; biotin, 0.04; and $Na_2SiO_3 \cdot 5H_2O$, 57 [19].

Microorganisms isolated from the algo–bacterial culture were grown under aerobic conditions on petri dishes with tenfold diluted fish peptone agar (FPA/10), as well as on the original medium with the algal extract (DA). To obtain the DA medium, the cells of *S. acus* were accumulated on a filter. Then, 1 g of the biomass was resuspended in 100 ml of water, brought to boiling, and supplemented with 1.5 g of agar. The initial algo–bacterial culture was inoculated into sterile medium. Bacterial cultures grew on petri dishes under aerobic conditions at 25°C; pure bacterial cultures were isolated on the same medium using the streak inoculation technique.

The physiological and biochemical properties of bacteria were determined by the standard methods [20, 21].

The amount of microalgal cells and the total number of bacterial cells colonizing *S. acus* were determined by epifluorescence microscopy of the preparations stained with DAPI (4,6-diamidino-2-phenylindole) (0.5 μg/ml); a known amount of the cell suspension was filtered through 0.2-μm Nucleopore (PC) membranes and examined under an Olympus epifluorescence microscope (Japan). Cell counting was performed using the software package developed at the Limnological Institute, Siberian Branch, Russian Academy of Sciences (inventor's certificate no. 2005610667).

The morphological properties of the obtained strains of microorganisms were determined by light microscopy, scanning electron microscopy (Philips SEM 525M; Netherlands), and transmission electron microscopy (Leo 906E Zeiss; Germany). The strains were identified to the species level using the manuals [22, 23].

DNA extraction and PCR. DNA from pure bacterial cultures was extracted using lysozyme, 10% SDS, and phenol–chloroform extraction. PCR amplification of the obtained DNA was performed using the Amplisens kit (Moscow, Russia) and the following universal primers for the 16S rRNA gene of eubacteria: 500L (5'-CGTGCCAGCAGCCGCGGTAA-3') and 1350R (5'-GACGGGCGGTGTGTACAAG-3'). The PCR cycle parameters were as follows: 94°C for 1 min, 94°C for 20 s, 50°C for 20 s, 72°C for 30 s (30 cycles), and 72°C for 5 min.

Determination of nucleotide sequences and phylogenetic analysis. Sequencing of the amplification products was performed on a CEQ8800 automatic sequencer (Beckman Coulter, United States) using the GenomeLab DTCS Quick Start Kit (Beckman Coulter, United States) and the above-mentioned primers. The obtained nucleotide sequences of the 16S rRNA genes (570 bp) were deposited in the GenBank under accession numbers EU671061–EU671073. The nucleotide sequences of the studied strains were analyzed using the BioEdit 5.0.9 software package; phylogenetic analysis was performed using the MEGA 3.1. software package.

RESULTS AND DISCUSSION

During cultivation of the laboratory culture of *S. acus*, inhibition of cell growth with the subsequent cell death was observed. Microscopic examination of DAPI-stained preparations revealed the accompanying microflora colonizing the diatom cells. In the course of the experiment, the number of bacterial cells increased from 1.2×10^5 to 5.4×10^6 cells/ml (Fig. 1).

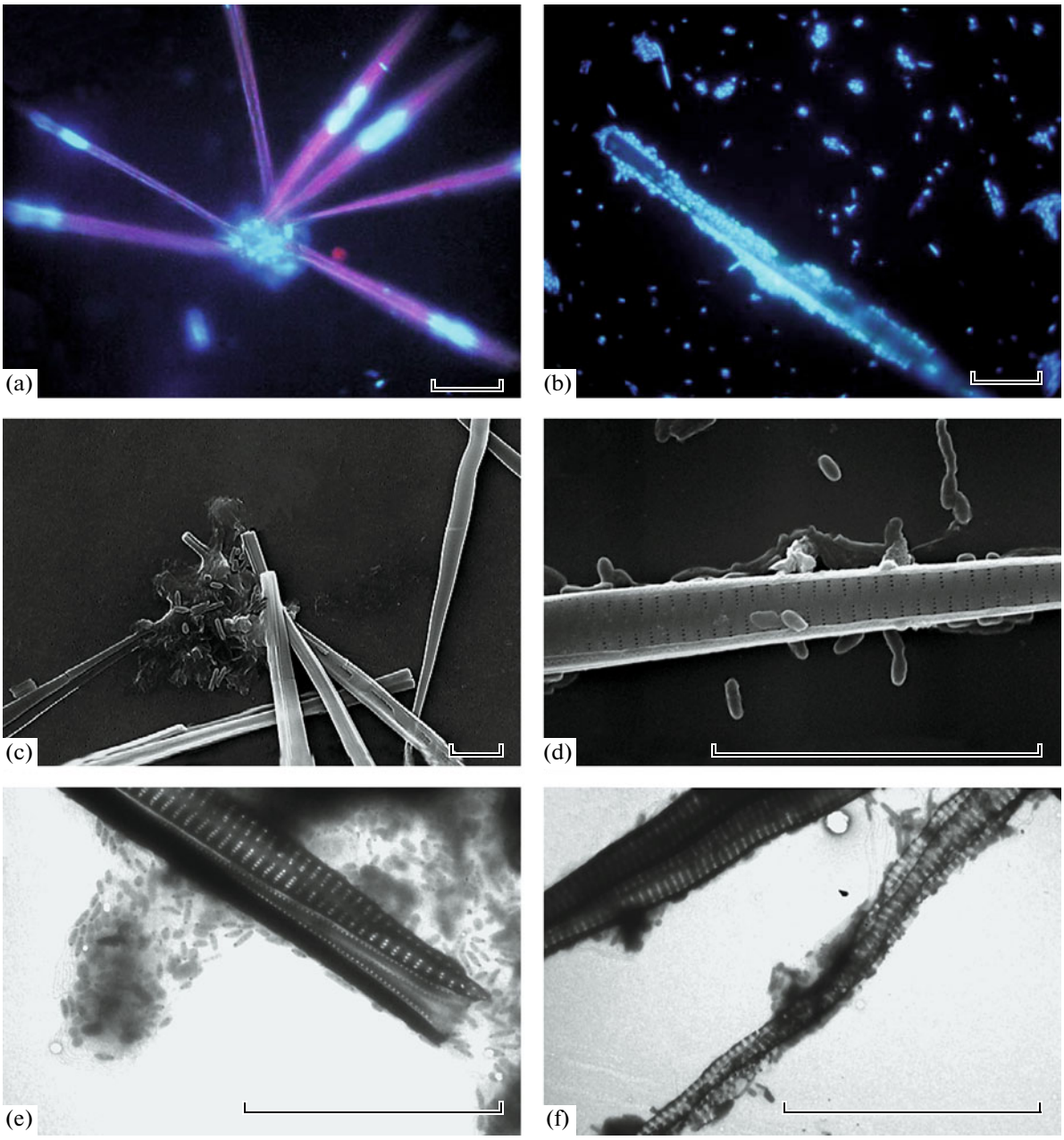

Fig. 2. Bacteria associated with the laboratory culture of *S. acus*. Localization of bacteria: (a) and (b) in the mucilage surrounding the living cells of *S. acus* (red fluorescence, chloroplasts; blue fluorescence, nuclei and nucleoids of DAPI-stained bacterial cells); (c) and (d) on the surfaces of *S. acus* cells; (e) inside dead *S. acus* cells; (f) on the frustules of the decomposed algal cells: (a) and (c) epifluorescence microscopy; (b) and (d) scanning electron microscopy; and (e) and (f) transmission electron microscopy. Scale bar: a, c, and d, 10 μm; b, 1 μm; e and f, 20 μm.

During the first 6 days, an increase in the numbers of both *S. acus* and bacterial cells was observed. The results of microscopic examination demonstrated that, during the first several days of cultivation, bacteria were localized in the mucilage surrounding the cells of living diatoms, in which chloroplasts and nuclei were clearly visible (Figs. 2a and 2b). During the stationary phase of microalgal growth (6–9 days), exponential growth of bacteria actively colonizing the surface of *S. acus* cells was observed (Figs. 2c and 2d). Subsequently (12–14 days of incubation), the concentration of *S. acus* cells decreased sharply, whereas the concentration of bacterial cells continued to increase, it was found that they actively colonized the surfaces of algal cells (Figs. 2c and 2d), penetrated dead diatoms (Fig. 2e), and localized on the frustules of decomposed algal cells (Fig. 2f).

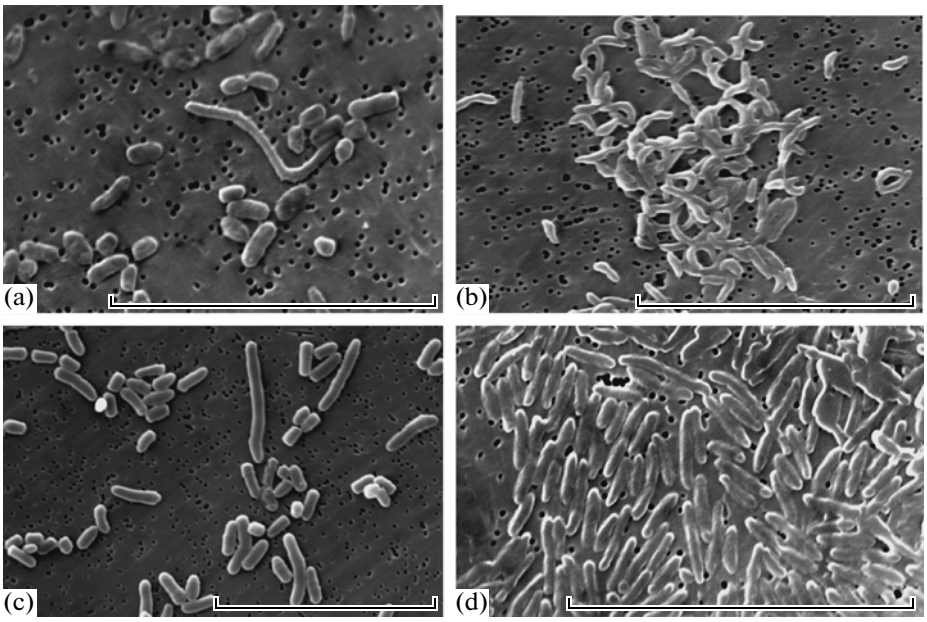

Fig. 3. Morphology of the cultivable bacteria isolated from the laboratory algal bacterial culture of *S. acus*: baik7s (a), baik37s (b), baik22s (c), and baik17s (d). Scanning electron microscopy. Scale bar, 10 μm.

From the *S. acus* at the stage of growth inhibition, 13 strains with different morphological (Figs. 3 and 4) and biochemical properties (table) were isolated on solid media and described. The strains baik7s, baik8s, baik14s, baik16s, baik21s, baik22s, baik25s, and baik26s were isolated on the FPA/10 medium; the strains baik17s, baik20s, baik27s, baik28s, and baik37s were isolated on the DA medium at 25–28°C and pH 6.8–7.0. The phylogenetic tree shows that the isolated strains are grouped into three clusters; the strains isolated on the DA medium form a separate cluster (Fig. 5).

On the basis of its morphological and biochemical properties, strain baik7s was assigned to the genus *Sphingomonas*. The cells are rod-shaped (0.4–0.5 × 0.8–1.0 μm) (Fig. 3a) and gram-negative. According to the results of comparative analysis of the obtained 16S rRNA gene sequences and all the known sequences in the GenBank database, strain baik7s displayed the highest similarity (92%) to *Novosphingobium* subterraneum (*Sphingomonas subterranea*) isolated from marine sediments (Fig. 5).

The cells of strain baik8s are straight or slightly gram-negative curved rods (0.4–0.5 × 1.2–1.5 μm) arranged individually or in pairs (Fig. 4a). According to the results obtained, the properties of strain baik8s correspond to the main properties of *Alcaligenes paradoxus* which, according to its taxonomic characteristics [22] was transferred into the genus *Variovorax* with a new name, *Variovorax paradoxus*. According to the data obtained by the 16S rRNA gene analysis (Fig. 5), strain baik8s showed a 100% similarity with the *V. paradoxus* strain isolated from plant rhizosphere.

A number of properties of the strains baik14s, baik16s, baik25s, and baik26s allowed us to identify them as *Pseudomonas fluorescens* [23]. The cells of these strains are rods (0.5–0.8 × 1.0–2.0), motile by means of several polar flagella (Figs. 4b and 4c). During growth on the medium, the cells excreted a fluorescent pigment. According to the results of analysis of the obtained 16S rRNA gene sequences (Fig. 5), strain baik14s displayed the highest similarity (99%) with the *P. fluorescens* strain isolated from river water; the similarity levels between the strains baik16s, baik25s, and baik26s and this *P. fluorescens* strain were slightly lower (98, 97, and 96%, respectively).

The morphological and biochemical properties of strain baik21s were similar to those of *Microbacterium trichothecenolyticum*. The cells are slightly curved rods (0.4–0.5 × 1.2–1.4 μm) (Fig. 4d), motile. Analysis of the 16S rRNA gene sequences (Fig. 5) indicated that strain baik21s was most closely related to *M. trichothecenolyticum* (99% homology) isolated among the symbionts of a *Chlorella* culture.

On the basis of its morphological and biochemical properties, strain baik22s was assigned to the genus *Rhodococcus*. The cells of a 1-day culture were rounded or ovoid and appear separately. In old cultures, cocci (0.4–0.8 μm) germinated into short rods (0.4–0.5 × 0.8–1.0 μm), which then became elongated (0.5 × 3.0–6.5 μm) and curved (Fig. 3d). According to the data obtained by the 16S rRNA gene analysis, strain baik22s showed 100% similarity to the strain of an unidentified species of the genus *Rhodococcus*, isolated from Antarctic water (Fig. 5).

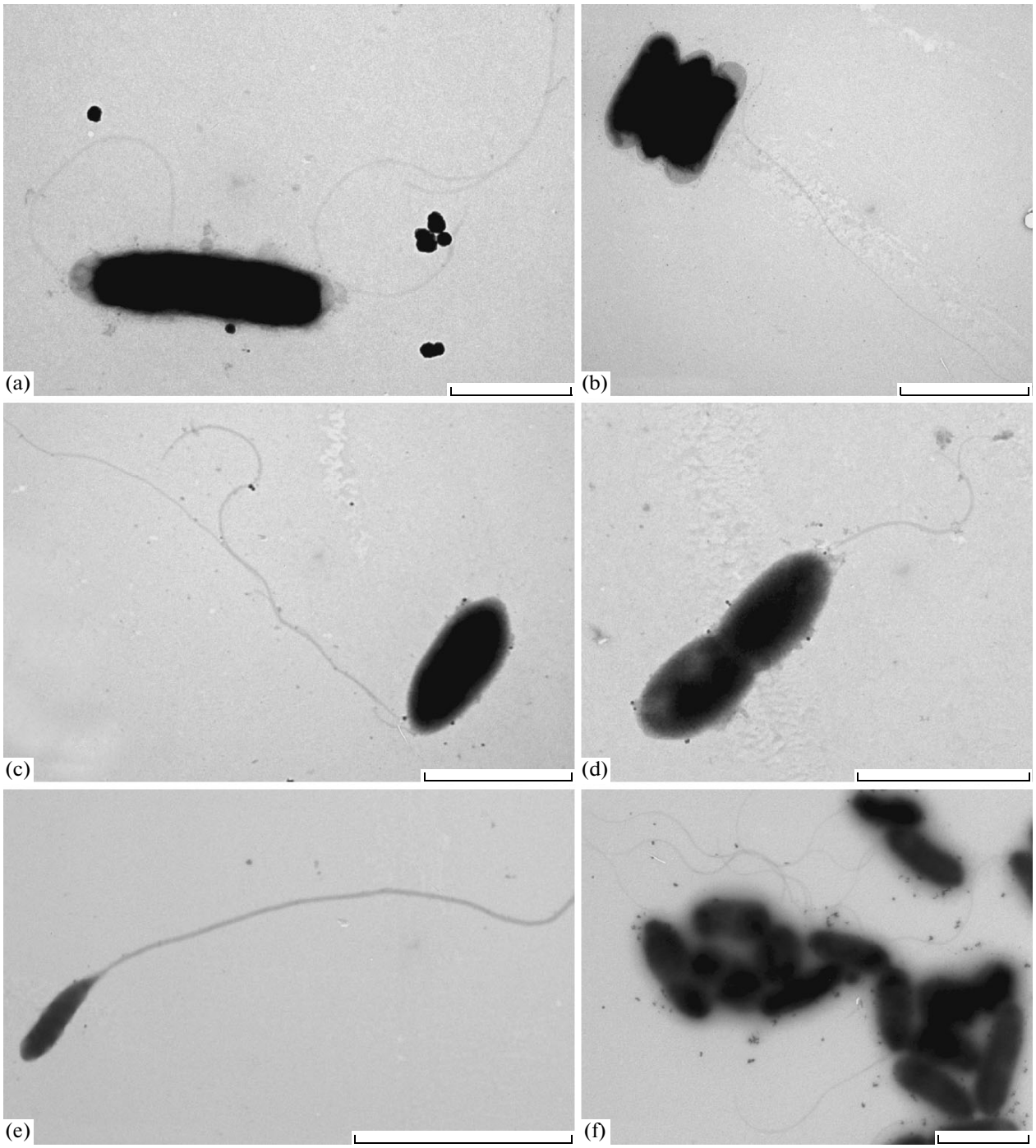

Fig. 4. Morphology of the cultivable bacteria isolated from the laboratory algal bacterial culture of *S. acus*: baik8s (a), baik14s (b), baik16s (c), baik21s (d), baik28s (e), and baik17s (f). Transmission electron microscopy. Scale bar: a, 1 μm; b-e, 2 μm; f, 5 μm.

The strains with similar cultural properties, which were isolated on DA medium, belonged to the *Caulobacter* and *Brevundimonas* morphotypes. On solid media, these strains formed very small (less than 1 mm), pinpoint, convex, transparent colonies. The cells of 1-day cultures of the strains baik28s and baik37s were vibrioid (0.2–0.4 × 1.0–2.5 μm) (Figs. 3b and 4e), gram-negative, with prosthecae, and are motile by means of flagella. The morphological and biochemical properties of the strains baik28s and strain baik37s were similar to those of *Caulobacter vibrioides*. According to the data obtained by the 16S rRNA gene analysis (Fig. 5), the strains baik28s and baik37s were clustered together with *Caulobacter tundrae* (91 and 93% similarities with baik28s and baik37s, respectively) and *Brevundimonas vesicularis*

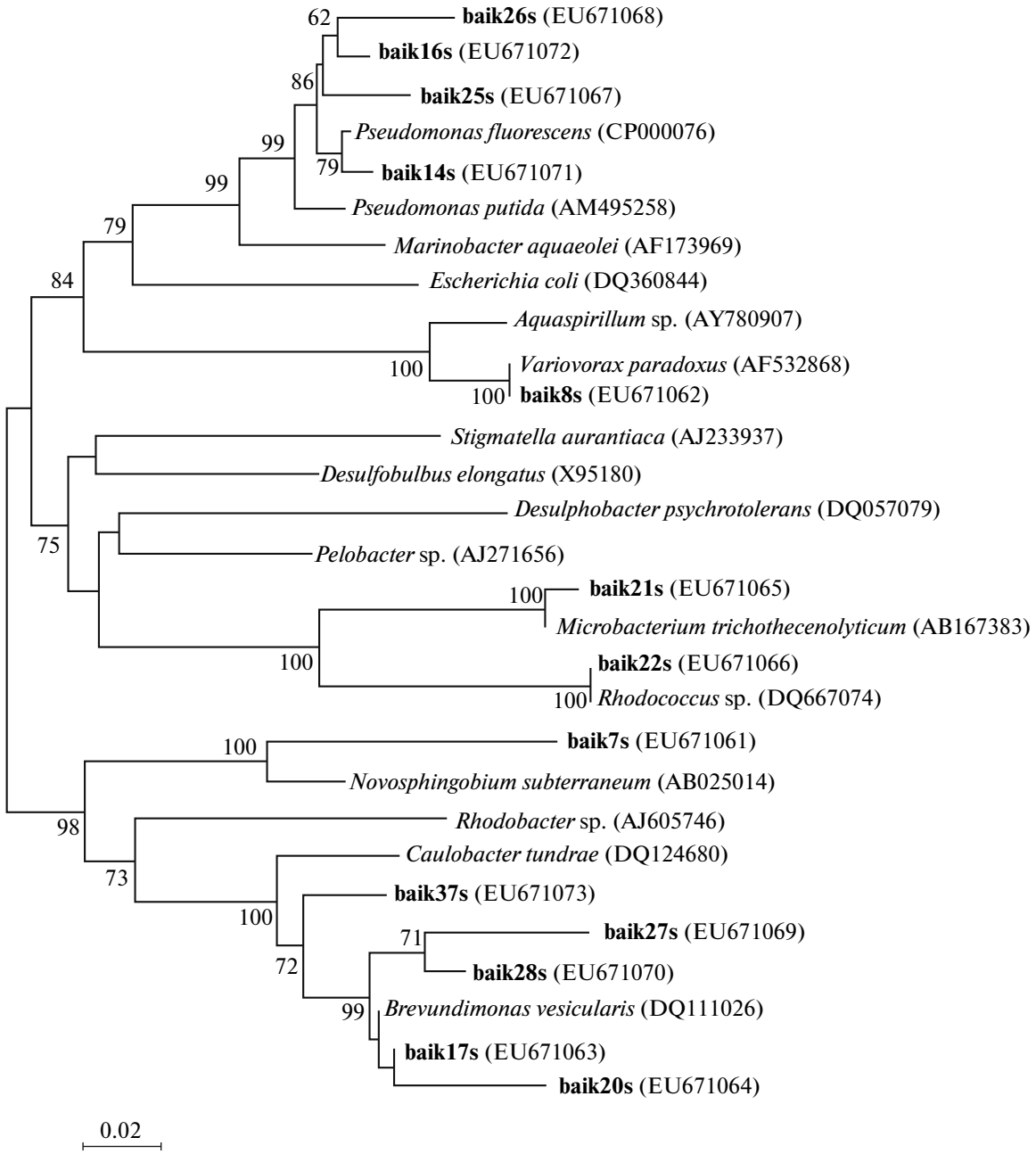

Fig. 5. Phylogenetic tree of bacterial strains associated with the diatom *S. acus* from Lake Baikal constructed on the basis of the analysis of the 16S rRNA gene sequences (570 bp) using the neighbor-joining method. The numerals at the branching points show the results of the bootstrap analysis. The sequences in bold were determined in this work.

strains (96 and 95% similarities with baik28s and baik37s, respectively). The cells of the strains baik17s, baik20s, and baik27s were bacteroids, rods with rounded ends (0.4–0.5 × 1.0–1.5 μm), motile by means of one to three flagella; prosthecae were not detected (Figs. 3d and 4f). The biochemical properties of the strains baik17s and baik20s were similar to those of *B. vesicularis*. According to the results of the 16S rRNA gene analysis (Fig. 5), the strains baik17s and baik20s were also closely related (99 and 95% similarities, respectively) to the described strain of *B. vesicularis* isolated from a freshwater basin in Germany. Analysis of the 16S rRNA gene sequence (Fig. 5) of strain baik27s revealed its similarity with *B. vesicularis* (92% homology) (Fig. 5).

It is well known that freshwater bacteria are able to grow in an algal culture in the absence of organic substrates, using polysaccharide secretory products of the diatoms as a sole carbon source [13]. Proteases, phosphatases, amylases, and lecithinases produced by bac-

Morphological and biochemical properties of the isolated strains

Property	Strain characteristics												
	baik7s	baik8s	baik14s	baik16s	baik17s	baik20s	baik21s	baik22s	baik25s	baik26s	baik27s	baik28s	baik37s
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+
Endospores	–	–	–	–	–	–	–	–	–	–	–	–	–
Gram reaction	–	–	–	–	–	–	+	+	–	–	–	–	–
Enzymatic activity:													
catalase	+	+	+	+	+	+	+	+	+	+	+	+	+
oxidase	+	+	+	+	+	+	+	+	+	+	+	–	–
gelatinase	+	+	–	–	+	+	+	+	–	–	–	–	–
lipase	–	–	–	–	–	–	–	–	–	–	–	–	–
lecithinase	+	+	+	+	+	+	–	+	–	–	–	–	–
phosphatase	+	+	+	+	+	+	+	+	+	+	+	+	+
caseinase	+	+	+	+	+	+	–	+	–	–	–	–	–
amylase	+	+	–	–	+	+	–	–	–	–	+	+	+
Voges–Proskauer reaction	–	–	–	+	+	+	–	–	+	+	+	+	+
Nitrate reduction	+	+	+	–	–	–	–	–	–	–	+	–	–
Growth at 6.5% NaCl	–	–	–	–	–	–	–	–	–	–	–	–	
Production of acids from carbohydrates:													
glucose	–	–	+	+	+	+	–	–	+	+	+	+	+
sucrose	–	–	+	–	–	–	–	–	–	–	–	+	+
xylose	+	–	+	+	–	–	+	+	+	+	–	+	+
arabinose	+	+	+	+	–	–	+	+	+	+	–	+	+
lactose	–	–	–	–	–	–	–	–	–	–	–	+	+
sorbitol	–	–	–	–	–	–	–	–	–	–	–	–	–
mannitol	+	–	–	–	+	+	+	–	–	–	–	–	–

teria can hydrolyze proteins, carbohydrates, and other polymers produced by diatoms, which results in the removal of the protective organic layer from the surfaces of their silica frustules, thereby hastening the process of their dissolution [12]. The isolated strains exhibited various enzymatic activities; the broadest range of enzymatic activities was exhibited by strains baik7s (*Sphingomonas* sp.), baik8s (*Variovorax paradoxus*), and baik17s (*Brevundimonas vesicularis*).

The proposed technique for bacteria cultivation on the medium with the algal extract allowed us to isolate oligotrophic bacteria belonging to the genera *Brevundimonas* and *Caulobacter* of the class *Alphaproteobacteria*, which have not been previously described as diatom satellites. It was shown that the cells of the *Brevundimonas* and *Caulobacter* morphotypes penetrate into the dead diatom cells and localize on the frustules of the decomposed algal cells (Figs. 2e and 2f).

The results of phylogenetic analysis have demonstrated that the isolated strains, which were associated with the freshwater diatom *S. acus* during its cultivation, were diverse, belonging to various taxonomic groups. Six strains (baik7s, baik17s, baik20s, baik27s, baik28s, and baik37s) belong to the class *Alphaproteobacteria*, one strain (baik8s) was affiliated to the *Betaproteobacteria*, four strains (baik14s, baik16s, baik25s, and baik26s) belong to the *Gammaproteobacteria*, and two strains (baik21s and baik22s) were affiliated to the *Actinobacteria*. Comparative analysis of the taxonomic diversity of the isolated pure cultures by traditional microbiological methods and molecular biological techniques made it possible to reveal with a high reliability the species diversity of the studied bacteria and affiliate them with the following taxa: *Sphingomonas* sp., *Variovorax paradoxus, Pseudomonas fluorescens, Microbacterium trichothecenolyticum, Rhodococcus* sp., *Caulobacter vibrioides*, and *Brevundimonas vesicularis*.

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REFERENCES

1. Croft, M.T., Lawrence, A.D., Raux-Deery, E., Warren, M.J., and Smith, A.G., Algae Acquire Vitamin B_{12} Through a Symbiotic Relationship with Bacteria, *Nature*, 2005, vol. 438, pp. 90–93.
2. Droop, M.R., Vitamins, Phytoplankton and Bacteria: Symbiosis or Scavenging, *J. Plankton Res.*, 2007, vol. 29, pp. 107–113.
3. Reinman, L., Steward, G.F., and Azam, F., Dynamics of Bacterial Community Composition and Activity During a Mesocosm Diatom Bloom, *Appl. Environ. Microbiol.*, 2000, vol. 66, pp. 578–587.
4. Kaczmarska, I., Erhman, J., Bates, S.S., Green, D.H., Le'ger, C., and Harris, J., Diversity and Distribution of Epibiotic Bacteria on *Pseudonitzschia multiseries* (Bacillariophyceae) in Culture, and Comparison with Those on Diatoms in Native Seawater, *Harmful Algae*, 2005, vol. 4, pp. 725–741.
5. Lau, W.W.Y., Keil, R.G., and Armbrust, V., Succession and Diel Transcriptional Response of the Glycolate-Utilizing Component of the Bacterial Community During a Spring Phytoplankton Bloom, *Appl. Environ. Microbiol.*, 2007, vol. 73, pp. 2440–2450.
6. Field, C.B., Behrenfeld, M.J., Randerson, J.T., and Falkonski, P., Primary Production of the Biosphere: Integrating Terrestrial and Oceanic Components, *Science*, 1998, vol. 281, pp. 237–240.
7. Bidle, K.D. and Azam, F., Accelerated Dissolution of Diatom Silica by Marine Bacterial Assemblages, *Nature*, 1999, vol. 397, pp. 508–512.
8. Bidle, K.D., Brzezinski, M.A., Long, R.A., Jones, J.L., and Azam, F., Diminished Efficiency in the Oceanic Silica Pump Caused by Bacteria-Mediated Silica Dissolution, *Limnol. Oceanogr.*, 2003, vol. 48, no. 5, pp. 1855–1868.
9. Terekhova, V.E., Aizdaicher, N.A., Karpenko, A.A., and Buzoleva, L.S., Investigation of Relationships between Marine Diatoms and the Bacterium *Listeria monocytogenes, Mikrobiologiya*, 2006, vol. 75, no. 1, pp. 90–93 [*Microbiology* (Engl. Transl.), viol 75, no. 1, pp. 73–76].
10. Fukami, K., Nishijima, T., and Ishida, Y., Stimulative and Inhibitory Effects of Bacteria on the Growth of Microalgae, *Hydrobiologia*, 1997, vol. 358, pp. 185–191.
11. Nicolas, J.-L., Corre, S., and Cocherd, J.-C., Bacterial Population Association with Phytoplankton Cultured in a Bivalve Hatchery, *Microbial Ecol*, 2003, vol. 48, pp. 400–413.
12. Bidle, K.D. and Azam, F., Bacterial Control of Silicon Regeneration from Diatom Detritus: Significance of Bacterial Ectohydrolases and Species Identity, *Limnol. Oceanogr.*, 2001, vol. 46, no. 7, pp. 1606–1623.
13. Bruckner, C.G., Bahulikar, R., Rahalkar, M., Schink, B., and Kroth, P.G., Bacteria Associated with Bentic Diatoms from Lake Constance: Phylogeny and Influences on Diatom Growth and Secretion of Extracellular Polymeric Substances, *Appl. Environ. Microbiol.*, 2008, vol. 74, pp. 7740–7749.
14. Kang, Y.H., Kim, J.D., Kim, B.H., Kong, D.S., and Ham, M.S., Isolation and Characterization of a Bio-Agent Antagonistic to Diatom *Stephanodiscus hantzschii, Appl. Microbiol.,* 2005, vol. 98, pp. 1030–1038.
15. Grachev, M.A., Denikina, N.N., Belikov, S.I., Likhoshway, Ye.V., Usol'tseva, M.V., Tikhonova, I.V., Adel'shin, R.V., Kler, S.A., and Shcherbakova, T.A., Elements of the Active Center of Silicon Transporters

in Diatoms, *Mol. Biol.*, 2002, vol. 36, no. 4, pp. 679–681 [*Molecular Biology* (Engl. Transl.), vol. 36, no. 4, pp. 534–537].

16. Shcherbakova, T.A., Masyukova, Yu.A., Safonova, T.A., Petrova, D.P., Vereshchagin, A.L., Minaeva, T.V., Adel'shin, R.V., Triboy, T.I., Stonik, I.V., Aizdaitcher, N.A., Kozlov, M.V., Likhoshway, Ye.V., and Grachev, M.A., Conserved Motif CMLD in Silicic Acid Transport Proteins of Diatoms, *Mol. Biol.*, 2005, vol. 39, no. 2, pp. 303–316 [*Molecular Biology* (Engl. Transl.), vol. 39, no. 2, pp. 269–280].
17. Grachev, M., Sherbakova, T., Masyukova, Y., and Likhoshway, Y., A Potential Zinc-Binding Motif in Silicic Acid Transport Proteins of Diatoms, *Diatom Res*, 2005, vol. 20, pp. 409–411.
18. Vereshchagin, A.L., Glyzina, O.Yu., Basharina, T.N., Safonova, T.A., Latyshev, N.A., Lyubochko, S.A., Korneva, E.S., Petrova, D.P., Annenkov, V.V., Danilovtseva, E.N., Chebykin, E.P., Volokitina, N.A., and Grachev, M.A., Cultivation of Freshwater *Synedra acus* in a 100-Liter Photobioreactor and assessment of the Biomass Composition, *Biotekhnol.*, 2008, no. 4, pp. 55–63.
19. *Culture Collection of Algae and Protozoa: Catalogue of strains*, Thompson, A.S., Rhodes, J.C., and Pettman, I., Eds., Kendal: Titus Wilson and Son, 1988.
20. Egorov, N.S., *Praktikum po mikrobiologii* (A Practical Course on Microbiology), Moscow: Mosk. Gos. Univ., 1976.
21. *Manual of Methods for General Bacteriology*, Gerhardt, P. et al., Eds., Washington: Amer. Soc. Microbiol., 1981 [Russ. Transl. Moscow: Mir, 1984].
22. *Bergey's Manual of Systematic Bacteriology*, 8th ed., vol.1–2, Holt, J.G., Ed, Baltimore–London: Williams and Wilkins, 1986.
23. *Bergey's Manual of Systematic Bacteriology, vol. I–II*, Garrity, G.M., Ed., New York: Springer-Verlag, 2005.