ISSN 0026-2617, Microbiology, 2013, Vol. 82, No. 6, pp. 721–727. © Pleiades Publishing, Ltd., 2013.
Original Russian Text © V.N. Akimov, O.A. Podosokorskaya, M.G. Shlyapnikov, V.F. Gal'chenko, 2013, published in Mikrobiologiya, 2013, Vol. 82, No. 6, pp. 707–714.

EXPERIMENTAL ARTICLES

Dominant Phylotypes in the 16S rRNA Gene Clone Libraries from Bacterial Mats of the Uzon Caldera (Kamchatka, Russia) Hydrothermal Springs

V. N. Akimov[a, b, 1], **O. A. Podosokorskaya**[b], **M. G. Shlyapnikov**[a], **and V. F. Gal'chenko**[b, 1]

[a] *Skryabin Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences,*
pr. Nauki 5, Pushchino, 142290 Russia
[b] *Winogradsky Institute of Microbiology, Russian Academy of Sciences,*
pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia

Received July 10, 2012

Abstract—In situ analysis of the 16S rRNA genes from bacterial mats of five hydrothermal springs (36–58°C) in the Uzon caldera (Kamchatka, Russia) was carried out using clone libraries. Eight clone libraries contained 18 dominant phylotypes (over 4–5%). In most clone libraries, the phylotype of the green sulfur bacterium *Chlorobaculum* sp. was among the dominant ones. The phylotypes of the green nonsulfur bacteria *Chloroflexus* and *Roseiflexus* and of purple nonsulfur bacteria *Rhodoblastus, Rhodopseudomonas*, and *Rhodoferax* were also among the dominant ones. Cyanobacteria were represented by one dominant phylotype in a single spring. Among nonphototrophic bacteria, the dominant phylotypes belonged to *Sulfyrihydrogenibium* sp., *Geothrix* sp., *Acidobacterium* sp., *Meiothermus* sp., *Thiomonas* sp., *Thiofaba* sp., and *Spirochaeta* sp. Three phylotypes were not identified at the genus level. Most genera of phototrophic and nonphototrophic organisms corresponding to the phylotypes from Uzon hydrotherms have been previously revealed in the hydrotherms of volcanically active regions of America, Asia, and Europe. These results indicate predominance of bacterial mats carrying out anaerobic photosynthesis in the hydrotherms of the Uzon caldera.

Keywords: Uzon volcanic caldera, hydrothermal springs, bacterial mats, clone library, 16S rRNA

DOI: 10.1134/S0026261713060027

Bacterial mats dominated by photosynthetic microorganisms (e.g., cyanobacteria in cyanobacterial mats) are common in terrestrial hydrotherms with temperatures below 72–73°C and have been best studied in volcanically active regions of North America, Asia, and Europe [1–3]. Depending on the physicochemical factors, the mats differ in the composition of photosynthetic and non-photosynthetic organisms, which results in differences in their color. Thus, cyanobacteria dominate in dark-green mats, *Roseiflexus* sp. bacteria dominate in orange ones, while light yellow or yellow-green mats may be dominated by anaerobic photosynthetic bacteria, cyanobacteria, or even non-photosynthetic microorganisms. Determination of the dominant organisms in bacterial mats of terrestrial hydrotherms is important for assessment of the biogeochemical potential of these communities, as well as of their structural and functional organization. Determination of the taxonomic diversity of microbial communities by cultural techniques (ex situ) is insufficient for the purpose, and molecular biological approaches (in situ) are presently used for the purpose. Analysis of the 16S rRNA clone libraries obtained from the DNA isolated from environmental samples is a usual approache to investigation of the taxonomic structure of microbial communities [3]. The works on cyanobacterial mats from two terrestrial hydrothermal springs in the Yellowstone National Park, United States, are an example of the high efficiency of molecular techniques [4–6].

We used clone libraries in order to determine the dominant phylotypes in bacterial mats from five hydrothermal springs with temperatures from 36 to 58°C, located at three thermal areas of the Uzon caldera, Kamchatka, Russia. Since cyanobacteria were not revealed as the dominant organisms in most of the mats, a more general term, bacterial mats, is used in the present work.

MATERIALS AND METHODS

Hydrothermal springs. Five small springs with water temperatures in the gryphon from 36 to 58°C and low flow rates not exceeding 1 L/min, which were used for sampling, were located at the East Field (three), Orange Field (one), and North Field (one) thermal areas.

---

[1] Corresponding author; e-mail: akimov@ibpm.pushchino.ru, valgalch@inmi.host.ru

Table 1. Sampling sites for the Uzon caldera bacterial mats

Spring location	Spring, gryphon temperature
East Field, brook shore at the Paryashchii Sapozhok area 54°29.942′ N, 160°00.677′ E	Yupiter, 46–58°C (according to thermal monitoring during 2007–2008)
East Field, Paryashchii Sapozhok area 54°29.976′ N, 160°00.679′ E	Fobos 37°C
Northern slope of the Lake Khloridnoe shore (between the Eastern and Central fields) 54°30.010′ N, 160°00.462′ E	Khloridnoe 1 36°C
Northern edge of the Orange Field 54°30.143′ N, 160°00. 049′ E	Oranzhevoe 1 45°C
Upper part of the North Field 54°30.675′ N, 160°00.310′ E	Severnoe 1 40°C

For all springs, the diameter of the water area around the gryphon did not exceed 1.0 m, with the depth not exceeding 0.2 m. Samples were collected in 2005–2008 from the mats formed in the cone of the spring along the perimeter of the water area (Table 1).

Sampling and storage. The samples were taken from the mats of yellowish-green and light green color, which were classified as belonging to types "olive" and "green" according to Gorlenko et al. [7, 8]. Mat thickness did not exceed 5 mm. Columns approx. 1 cm^3 in volume were excised, placed in 2-mL plastic tubes, and fixed with ethanol (final concentration 25–30%). Fixed samples were transported to the laboratory at 10–30°C and then stored at –20°C.

DNA isolation. Prior to isolation, the cells were collected by centrifugation and washed twice with TE buffer containing 50 mM EDTA (10 mM Tris–HCl, 50 mM EDTA, pH 8.0). The cells were disintegrated by three repeating cycles of freezing (–70°C) and thawing (50°C), followed by heating for 5 min at 85°C [9]. DNA was isolated by the phenol method [10].

The 16S rRNA gene was amplified with the universal eubacterial primers 27f (5'-AGAGTTTGATCCTGGCTCAG) and 1492r (5'-TACGGYTACCTTGTTACGACTT); the universal archaeal primers A8f (5'-TCCGGTTGATCCTGCCGG), A800r (5'-GTTTAC(R)-GCC(R)GGACTAC), and A1041r (5'-GGCCATGCACC(W)CCTCTC); and cyanobacterial primers Cya106F (5'-CGGACGGGTGAGTAACGCGTGA), Cya359F (5'-GGGGAATTTTCCCGCAATGGG), Cya781R(a) (5'-GACTACTGGGGTATCTAATCCCATT), and Cya781R(b) (5'-GACTACAGGGGTATCTAATCCCTTT). PCR was carried out on GeneAmp PCR System 2700 (Applied Biosystems, United States).

Cloning. PCR fragments were cloned in the pGEM-T vector (Promega, United States). Screening of a clone library began with amplification of the cloned 16S rRNA genes with the primers T7f (5'-TAATACGACTCACTATA) and Sp6r (5'-TATTTAGGTGACACTATAG), specific to the relevant promoter sites of the plasmid, with subsequent reamplification of the insert with eubacterial primers 27f and 1492r. Restriction analysis of the PCR products using the *Hae*III and *Hha*I endonucleases was used to reveal the clones bearing similar DNA fragments [11]. The 16S rRNA gene fragments of at least 1100 nucleotides were sequenced for members of the clone groups differing according to the results of restriction analysis. The 16S rRNA gene clones were grouped into phylotypes according to the results of restriction analysis or the similarity between the nucleotide sequences exceeding 98%.

Nucleotide sequences of the 16S rRNA genes were determined on a CEQ2000 XL automatic sequencer (Beckman Coulter, United States) according to the manufacturer's recommendations. The nucleotide sequences were deposited to GenBank; their accession numbers are listed in Table 2.

Phylogenetic analysis. To determine the closely related organisms, the nucleotide sequences were compared to 16S rRNA gene sequences from the GenBank database (http://www.ncbi.nlm.nih.gov/genbank) and the ribosomal database (RDPII—http://rdp.cme.msu.edu). The sequences were aligned using the CLUSTAL W software package [12]. Phylogenetic trees were constructed using the TREECON software package [13].

RESULTS AND DISCUSSION

Characterization of the hydrothermal springs and bacterial mats studied. Most hydrothermal springs in the Uzon caldera were small springs with the diameter of the water area surrounding the gryphon ~1.0 m or less, with the water temperature below 70°C, and flow rates ~1 L/min or less. Bacterial mats (0.2–2.0 m^2) were located along the perimeter of the water area surrounding the gryphon or along the outflow channel. According to the color of the surface layer, three types of bacterial mats were discernible: yellow-green, green, and orange. Yellow-green mats corresponded to

Table 2. Taxonomic position of the phylotypes retrieved from eight 16S rRNA gene clone libraries of bacterial mats from the Uzon caldera, 2005–2008

Phylotype designation in the text	GenBank accession no.	Closest taxon	Phylum/class
Belong to genera of photosynthetic organisms			
Phylotype Uzon 1	KF614124	*Chlorobaculum*	*“Chlorobi”*
Phylotype Uzon 2	KF614125	*Chloroflexus*	*“Chloroflexi”*
Phylotype Uzon 3	KF614126	*Roseiflexus*	*“Chloroflexi”*
Phylotype Uzon 4	KF614127	*Rhodoferax*	*Proteobacteria/Betaproteobacteria*
Phylotype Uzon 5	KF614140	*Rhodoblastus*	*Proteobacteria/Alphaproteobacteria*
Phylotype Uzon 6	KF614128	*Rhodopseudomonas*	*Proteobacteria/Alphaproteobacteria*
Phylotype Uzon 7	KF614141	*Oscillatoriales*	*“Cyanobacteria”*
Do not belong to genera of photosynthetic organisms			
Phylotype Uzon 8	KF614129	*Geothrix*	*“Acidobacteria”*
Phylotype Uzon 9	KF614131	*Acidobacterium*	*“Acidobacteria”*
Phylotype Uzon 10	KF614131	*Sulfurihydrogenibium*	*“Aquificae”*
Phylotype Uzon 11	KF614132	*Meiothermus*	*“Deinococcus-Thermus”*
Phylotype Uzon 12	KF614133	*Thiomonas*	*Proteobacteria/Betaproteobacteria*
Phylotype Uzon 13	KF614134	*Thiofaba*	*Proteobacteria/Gammaproteobacteria*
Phylotype Uzon 14	KF614135	*Spirochaeta*	*“Spirochaetes”*
Phylotype Uzon 15	KF614136	*Calditerrivibrio*	*“Deferribacteres”*
Cannot be assigned to known genera			
Phylotype Uzon 16	KF614137	*Rhodobacteraceae*	*Proteobacteria/Alphaproteobacteria*
Phylotype Uzon 17	KF614138	*Thiotrichaceae*	*Proteobacteria/Gammaproteobacteria*
Phylotype Uzon 18	KF614139	*Thermotogae*	*“Thermotogae”*

the “olive” type (temperature zone of development 50–62°C), while the green ones corresponded to the “green” type (temperature zone of development 30–55°C) according to the classification by Gorlenko et al., who investigated the Termofil’nyi spring of the Uzon caldera [7, 8]. Some other springs with the same outflow area contained all types of mats located along the temperature gradient. Only one mat type, depending on the spring temperature and other abiotic factors, usually prevails in small springs. Yellow-green and light green mats occur throughout the Uzon caldera within a broad temperature range, from 30 to 60°C, while dark green and orange mats are less common. Yellow-green mats have been described and studied in the hydrotherms of Yellowstone National Park (United States), Iceland, New Zealand, and Japan [3, 17]. Light green mats, however, have not been investigated as a special subject.

The current work presents the results of investigation of yellow-green and light green mats, which are considered as a single group due to the indistinct difference in the color of the surface layer and the absence of other differentiating characteristics currently known, probably except for the presence of sulfur. The samples were collected from the mats formed along the perimeter of the water area around the gryphon. In small springs the water temperature along the perimeter of the water area is only several degrees lower than in the gryphon; thus, the temperature at the sampling site was not measured. Considerable diurnal variation of the water temperature in the gryphon was found (unpublished data on thermal monitoring of over 30 hydrothermal springs in the Uzon caldera).

Table 3. Dominant phylotypes in the 16S rRNA gene clone libraries of bacterial mats from the Uzon caldera

Spring	Phylotype	Frequency of occurrence in clone library, %		
		2005	2006	2008
Yupiter 46–58°C				
"Hot" zone (yellow-green mat)	Phylotype Uzon 10 (*Sulfurihydrogenibium* sp.)	70	90	
	Phylotype Uzon 2 (*Chloroflexus* sp.)	10		
"Warm" zone (green mat)	Phylotype Uzon 4 (*Rhodoferax* sp.)	10		
	Phylotype Uzon 15 (*Calditerrivibrio* sp.)	10	10	
	Phylotype Uzon 1 (*Chlorobaculum* sp.)	70		20
	Phylotype Uzon 7 (*Oscillatoriales*)	10		60
	Phylotype Uzon 3 (*Roseiflexus* sp.)	10		
	Phylotype Uzon 2 (*Chloroflexus* sp.)			
	Phylotype Uzon 11 (*Meiothermus* sp.)			20
		10		
Fobos 37°C	Phylotype Uzon 1 (*Chlorobaculum* sp.)	45		
	Phylotype Uzon 3 (*Rhodoferax* sp.)	30		
	Phylotype Uzon 6 (*Rhodopseudomonas* sp.)	25		
Khloridnoe 1 36°C	Phylotype Uzon 12 (*Thiomonas* sp.)	30		
	Phylotype Uzon 5 (*Rhodoblastus* sp.)	20		
	Phylotype Uzon 1 (*Chlorobaculum* sp.)	20		
	Phylotype Uzon 8 (*Geothrix* sp.)	20		
	Phylotype Uzon 9 (*Acidobacterium* sp.)	10		
Oranzhevoe 1 45°C	Phylotype Uzon 13 (*Thiofaba* sp.)		50	
	Phylotype Uzon 1 (*Chlorobaculum* sp.)		25	
	Phylotype Uzon 14 (*Spirochaeta* sp.)		20	
	Phylotype Uzon 18 (*Thermotogae*)		5	
Severnoe 1 40°C	Phylotype Uzon 2 (*Chloroflexus* sp.)			40
	Phylotype Uzon 16 (*Rhodobacteraceae*)			30
	Phylotype Uzon 17 (*Thiotrichaceae*)			30

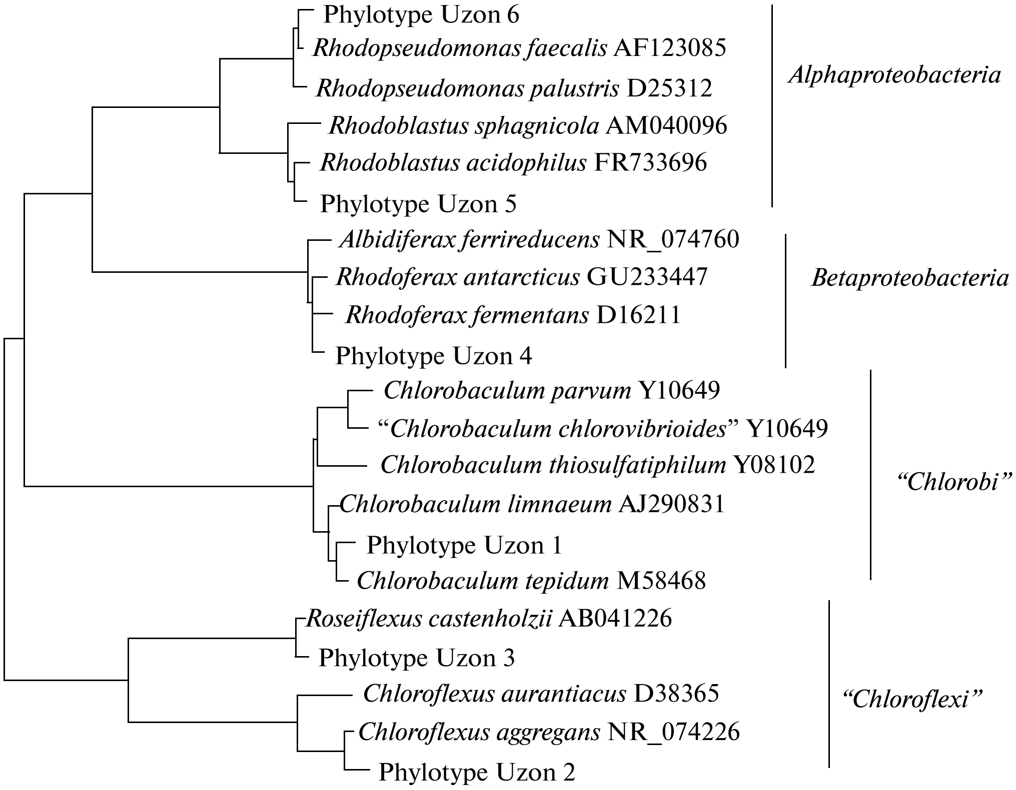
The diurnal temperature range is listed in Table 1 only for the Yupiter spring.

Specifics of 16S rRNA gene analysis in mat samples. The results of molecular biological analysis of microbial communities have repeatedly been reported to depend on the method of DNA isolation [14]. The phenol method used in the present work is often used for DNA isolation from environmental objects and is suitable for archaeal, bacterial, and eukaryotic cells, but not for endospores. We have previously shown good correlation between the results of in situ phylogenetic analysis (detected dominant phylotypes) obtained using this method of DNA isolation, and ex situ phylogenetic analysis (isolated dominant microorganisms) in the case of the mats formed by colorless sulfur bacteria [15].

Clone libraries were obtained only from the amplicons with the universal bacterial primers 27f and 1492r. The amplicons obtained with the universal archaeal 16S rRNA primers were revealed by electrophoresis in insignificant amounts and were not used to construct clone libraries.

Screening of the clone libraries was carried out on 30–70 clones for each library. Since in each sampling the dominant 3–6 phylotypes were represented by at least 80% of the clones, such limited sampling was sufficient for determination of the dominant phylotypes. The rounded values of the shares of the dominant phylotypes are listed in Table 3 (the minor phylotypes were not considered).

Identification of the phylotypes revealed by screening of the clone libraries by restriction analysis of the 16S rRNA gene amplicons was carried out using the sequenced fragments of 16S rRNA gene amplicons at least 1100 bp long. For each phylotype, most closely related organisms in the GenBank and RDP II data-



Phylogenetic tree constructed based on the 16S rRNA gene sequences and showing the position of uncultured bacteria revealed in the clone libraries among most closely related cultured photosynthetic bacteria. Phylotype Uzon 7 (an uncultured cyanobacterium) is not presented, since accurate determination of its generic position proved impossible.

bases were determined using the 97–98% and 94–95% threshold similarity values for the hypothetical species and genus identification, respectively. Genus identification of the phylotypes was ascertained by phylogenetic analysis with construction of phylogenetic trees, including all type strains of related genera. For the phylotypes without clear genus identification, the closest suprageneric taxon was specified. Since the similarity between the 16S rRNA gene sequences of the phylotypes and the GenBank sequences did not exceed 98%, only generic and suprageneric taxa of the closely related organisms are listed in Table 2.

Dominant phylotypes in the studied bacterial mats. The taxonomic position of all 18 dominant phylotypes revealed in eight 16S rRNA clone libraries obtained from yellow-green and light green mats of five springs studied is listed in Table 2. Genus identification was carried out for 15 out of 18 phylotypes, making it possible to consider 7 and 8 phylotypes as photosynthetic and non-photosynthetic organisms, respectively. While all 7 phylotypes representing photosynthetic bacteria exhibited over 97% 16S rRNA similarity with closely related photosynthetic bacteria (figure), it was not possible to carry out the genus identification for cyanobacterial phylotype Uzon 7. Because only the most closely related suprageneric taxa could be specified for the phylotypes Uzon 16–Uzon 18, their metabolic characteristics cannot be discussed.

Most of the phylotypes listed in Table 2 either represent bacterial genera containing the species isolated from hydrothermal springs [16–24], or members of these genera have been revealed in the springs by cultural [7, 8, 24–26] and/or molecular techniques [25–28, 32]. Only for the genus *Rhodoblastus*, no data are available concerning its occurrence in hydrothermal springs.

Diversity of yellow-green and light green bacterial mats according to the 16S rRNA gene analysis. Phylotype Uzon 1, closely related to the cyanobacterium *Chlorobaculum tepidum*, was retrieved from the mats of four out of five springs. This organism was originally isolated from New Zealand hydrothermal springs as *Chlorobium* sp. [17] and was described as *Chlorobium tepidum* [18], and subsequently reclassified as *Chlorobaculum tepidum* [19]. This was the only phototrophic microorganism in yellow-green and green bacterial mats of New Zealand hot springs, where cyanobacteria were not detected [17]. Green nonsulfur bacteria of the genus *Chloroflexus* [29] and purple nonsulfur photosynthetic bacteria of the genus *Chromatium* [30] have also been reported as the only phototrophic organisms in bacterial mats.

It was only in one out of five springs that the cyanobacterial phylotype Uzon 7 was revealed among the dominant forms (Table 3). Application of group-specific cyanobacterial primers for the 16S rRNA gene [31] revealed, however, the presence of cyanobacteria as minor phylotypes in the other springs studied, except for Khloridnoe 1 and Severnoe 1.

Non-photosynthetic organisms were mostly represented by the phylotypes belonging to sulfur-oxidizing bacterial genera: *Spirochaeta, Sulfurihydrogenibium, Thiofaba*, and *Thiomonas*.

Possible metabolic strategies for the organisms of the investigated mats. Most of the dominant phylotypes belonged to well-studied bacterial genera with known metabolism. Yellow-green and light green belong to the most widespread mat types in the Uzon caldera hydrothermal springs. Mats with predominance of green sulfur bacteria (*Chlorobaculum*) were originally reported in New Zealand springs [17]. They were found in the springs of the Yellowstone National Park, albeit rarely [2, 34], since high level of sulfide (suppressing cyanobacterial growth) and low pH (suppressing the growth of purple bacteria) are the conditions optimal for their development [2]. Our data obtained for five springs with temperatures from 36 to 58°C make it possible to suggest that bacterial mats with oxygenic photosynthesis (cyanobacterial mats) occur in a limited number of the Uzon caldera springs, while anoxygenic phototrophic communities prevail, dominated by green sulfur bacteria (*Chlorobaculum*), green nonsulfur bacteria (*Chloroflexus, Roseiflexus*), and purple nonsulfur bacteria (members of some genera within the classes *Alphaproteobacteria* and *Betaproteobacteria*) (Table 3). Bacterial mats in the Yupiter spring were investigated during 2005–2008, which made it possible to reveal the qualitative and quantitative variations in the composition of the dominant phylotypes. Yellow-green and light green bacterial mats are dynamic communities with a broad spectrum of photosynthetic microorganisms.

ACKNOWLEDGMENTS

This work was supported by the Russian Foundation for Basic Research, projects nos. 07-05-00153-a, 08-04-00164-a, 09-04-10113-k, and 10-04-10127-k.

REFERENCES

1. Gorlenko, V.M., Anoxygenic phototrophic bacteria, in *Proc. Winogradsky Inst. Microbiol.,* vol. 15, *Photosynthetic Bacteria*, Gal'chenko, V.F., Ed., Moscow: MAKS Press, 2010, pp. 133–174.
2. Madigan, M.T., Jung, D.O., Karr, E.A., Sattley, W.M., Achenbach, L.A., and van der Meer, M.T.J., Diversity of anoxygenic phototrophs in contrasting extreme environments, in *Geothermal Biology and Geochemistry in Yellowstone National Park*, Inskeep, W.P. and McDermott, T.R., Eds., Bozeman, MT: Montana State Univ. Thermal Biol. Inst., 2005, pp. 203–219.
3. Ward, D.M. and Cohan, F.M., Microbial diversity in hot spring cyanobacterial mats: pattern and prediction, in *Geothermal Biology and Geochemistry in Yellowstone National Park*, Inskeep, W.P. and McDermott, T.R., Eds., Bozeman, MT: Montana State Univ. Thermal Biol. Inst., 2005, pp. 185–202.
4. Ferris, M.J. and Ward, D.M., Seasonal distributions of dominant 16S rRNA-defined populations in a hot spring microbial mat examined by denaturing gradient gel electrophoresis, *Appl. Environ. Microbiol.*, 1997, vol. 63, pp. 1375–1381.
5. Allewalt, J.P., Bateson, M.M., Revsbech, N.P., Slack, K., and Ward, D.M., Effect of temperature and light on growth of and photosynthesis by *Synechococcus* isolates typical of those predominating in the Octopus Spring microbial mat community of Yellowstone National Park, *Appl. Environ. Microbiol.*, 2006, vol. 72, pp. 544–550.
6. van der Meer, M.T.J., Klatt, C.G., Wood, J., Bryant, D.A., Bateson, M.M., Lammerts, L., Schouten, S., Sinninghe Damste, J.S., Madigan, M.T., and Ward, D.M., Cultivation and genomic, nutritional, and lipid biomarker characterization of *Roseiflexus* strains closely related to predominant in situ populations inhabiting Yellowstone hot spring microbial mats, *J. Bacteriol.*, 2010, vol. 192, pp. 3033–3042.
7. Gorlenko, V.M., Bonch-Osmolovskaya, E.A., Kompantseva, E.I., and Starynin, D.A., Differentiation of microbial communities in connection with a change in the physicochemical conditions in thermophile spring, *Microbiology* (Moscow), 1987, vol. 56, pp. 250–257.
8. Gorlenko, V.M., Starynin, D.A., Bonch-Osmolovskaya, E.A., and Kachalkin, V.I., Production processes in microbial cenoses of the Thermofil'nyi Hot Spring *Microbiology* (Moscow), 1987, vol. 56, pp. 692–697.
9. Bej, A.K., Mahbubani, M.H., Dicesare, J.L., and Atlas, R.M., Polymerase chain reaction-gene probe detection of microorganisms by using filter-concentrated samples, *Appl. Environ. Microbiol*, 1991, vol. 57, pp. 3529–3534.
10. Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A., and Struhl, K., *Current Protocols in Molecular Biology*, New York: Wiley, 1994.
11. Alexandrov, A., Kaushik, D., and Pascal, S.M., Streamline method to analyze 16S rRNA gene clone libraries, *BioTechniques*, 2001, vol. 30, pp. 938–944.
12. Thompson, J.D., Higgins, D.G., and Gibson, T.J., CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix choice, *Nucleic Acids Res.,* 1994, vol. 22, pp. 4673–4680.
13. Van de Peer, Y. and De Wachter, R., TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment, *Comput. Appl. Biosci.*, 1994, vol. 10, pp. 569–570.
14. Salonen, A., Nikkilä, J., Jalanka-Tuovinen, J., Immonen, O., Rajilić-Stojanović, M., Kekkonen, R.A., Palva, A., and de Vos, W.M., Comparative analysis of fecal DNA extraction methods with phylogenetic microarray: effective recovery of bacterial and archaeal DNA

using mechanical cell lysis, *J. Microbiol. Methods*, 2010, vol. 81, pp. 127–134.

15. Chernousova, E.Yu., Akimov, V.N., Gridneva, E.V., Dubinina, G.A., and Grabovich, M.Yu., Phylogenetic in situ/ex situ analysis of a sulfur mat microbial community from a thermal sulfide spring in the North Caucasus, *Microbiology* (Moscow), 2008, vol. 77, pp. 219–223.
16. Castenholz, R.W., Bauld, J., and Jørgenson, B.B., Anoxygenic microbial mats of hot springs: thermophilic *Chlorobium* sp., *FEMS Microbiol. Lett.*, 1990, vol. 74, pp. 325–336.
17. Wahlund, T.M., Woese, C.R., Castenholz, R.W., and Madigan, M.T., A thermophilic green sulfur bacterium from New Zealand hot springs, *Chlorobium tepidum* sp. nov., *Arch. Microbiol.*, 1991, vol. 156, pp. 81–90.
18. Imhoff, J.F., Phylogenetic taxonomy of the family *Chlorobiaceae* on the basis of 16S rRNA and *fmo* (Fenna–Matthews–Olson protein) gene sequences, *Int. J. Syst. Evol. Microbiol.*, 2003, vol. 53, pp. 941–951.
19. Hanada, S., Takaichi, S., Matsuura, K., and Nakamura, K., *Roseiflexus castenholzii* gen. nov., sp. nov., a thermophilic, filamentous, photosynthetic bacterium that lacks chlorosomes, *Int. J. Syst. Evol. Microbiol.*, 2002, vol. 52, pp. 187–193.
20. O'Neill, A.H., Liu, Y., Ferrera, I., Beveridge, T.J., and Reysenbach, A.L., *Sulfurihydrogenibium rodmanii* sp. nov., a sulfur-oxidizing chemolithoautotroph from the Uzon Caldera, Kamchatka Peninsula, Russia, and emended description of the genus *Sulfurihydrogenibium, Int. J. Syst. Evol. Microbiol.*, 2008, vol. 58, pp. 1147–1152.
21. Vesteinsdottir, H., Reinisdottir, D.B., and Örlygsson, N.J., *Thiomonas islandica* sp. nov., a moderately thermophilic hydrogen- and sulfur-oxidizing betaproteobacterium isolated from a hot spring, *Int. J. Syst. Evol. Microbiol.*, 2011, vol. 61, pp. 132–137.
22. Mori, K. and Suzuki, K., *Thiofaba tepidiphila* gen. nov., sp. nov., a novel obligately chemolithoautotrophic, sulfur-oxidizing bacterium of the *Gammaproteobacteria* isolated from a hot spring, *Int. J. Syst. Evol. Microbiol.*, 2008, vol. 58, pp. 1885–1891.
23. Aksenova, H.Y., Rainey, F.A., Janssen, P.H., Zavarzin, G.A., and Morgan, H.W., *Spirochaeta thermophila* sp. nov., an obligately anaerobic, polysaccharolytic, extremely thermophilic bacterium, *Int. J. Syst. Bacteriol.*, 1992, vol. 42, pp. 175–177.
24. Pohlschroeder, M., Leschine, S.B., and Canale-Parola, E., *Spirochaeta caldaria* sp. nov., a thermophilic bacterium that enhances cellulose degradation by *Clostridium thermocellum, Arch. Microbiol.*, 1994, vol. 161, pp. 17–24.
25. Gorlenko, V.M., Kompantseva, E.I., and Puchkova, N.N., Influence of temperature on prevalence of phototrophic bacteria in hot springs, *Microbiology* (Moscow), 1985, vol. 54, pp. 681–685.
26. Bateson, M.M., Wieland, A., and Ward, D.M., Microscopic examination of distribution and phenotypic properties of phylogenetically diverse *Chloroflexaceae*-related bacteria in hot spring microbial mats, *Appl. Environ. Microbiol.*, 2002, vol. 68, pp. 4593–4603.
27. Marcel, T.J., van der Meer, M.T.J., Klatt, C.G., Wood, J., Bryant, D.A., Bateson, M.M., Lammerts, L., Schouten, S., Damsté, J.S.S., Madigan, M.T., and Ward, D.M., Cultivation and genomic, nutritional, and lipid biomarker characterization of *Roseiflexus* strains closely related to predominant in situ populations inhabiting Yellowstone hot spring microbial mats, *J. Bacteriol.*, 2010, vol. 192, pp. 3033–3042.
28. Hall, J.R., Mitchell, K.R., Jackson-Weaver, O., Kooser, A.S., Cron, B.R., Crossey, L.J., and Takacs-Vesbach, C.D., Molecular characterization of the diversity and distribution of a thermal spring microbial community by using rRNA and metabolic genes, *Appl. Environ. Microbiol.*, 2008, vol. 74, pp. 4910–4922.
29. Giovannoni, S.J., Ward, D.M., Pevsbech, N.P., and Castenholz, R.W., Obligately phototrophic *Chloroflexus*: primary production in anaerobic, hot spring microbial mats, *Arch. Microbiol.*, 1987, vol. 147, pp. 80–87.
30. Madigan, M.T., *Chromatium tepidum* sp. nov., a thermophilic photosynthetic bacterium of the family *Chromatiaceae, Int. J. Syst. Bacteriol.*, 1986, vol. 36, pp. 222–227.
31. Nubel, U., Garcia-Pichel, F., and Muyzer, G., PCR primers to amplify 16S rRNA genes from Cyanobacteria, *Appl. Environ. Microbiol.*, 1997, vol. 63, pp. 3327–3332.
32. Gumerov, V.M., Mardanov, A.V., Beletsky, A.V., Bonch-Osmolovskaya, E.A., and Ravin, N.V., Molecular analysis of microbial diversity in the Zavarzin Spring, Uzon Caldera, Kamchatka, *Microbiology* (Moscow), 2011, vol. 80, pp. 244–251.
33. Kubo, K., Knittel, K., Amann, R., Fukui, M., and Matsuura, K., Sulfur-metabolizing bacterial populations in microbial mats of the Nakabusa hot spring, *Syst. Appl. Microbiol.*, 2011, vol. 34, pp. 293–302.
34. Bedard, D.L., Jerzak, G.V.S., Nübel, U., Bateson, M.M., and Ward, D.M., Novel thermophilic green sulfur bacteria discovered in hot springs in two regions of Yellowstone National Park, *Amer. Soc. Microbiol., General Meeting, Salt Lake City, Utah, 2002*, Abstract no. 159.

Translated by P. Sigalevich