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EXPERIMENTAL ARTICLES

The Search for Sulfate-Reducing Bacteria in Mat Samples from the Lost City Hydrothermal Field by Molecular Cloning

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Abstract—The work is dedicated to searching for microorganisms of the domain *Bacteria* capable of dissimilatory sulfate reduction in the samples of microbial mats from a carbonate chimney in the Lost City hydrothermal field. Cloning of 16S rRNA genes, the universal phylogenetic marker, and *dsrAB*, the functional marker for sulfate reduction, revealed phylotypes related to spore-forming *Desulfotomaculum*. No members of the *Deltaproteobacteria*, comprising the most numerous bacterial group with demonstrated capacity for dissimilatory sulfate reduction, were found. The phylogenetic position of 16S rRNA clones from the mats suggests that this microbial community is a unique consortium, where the energy flow is related to hydrogen of hydrothermal origin, while mass growth of primary produces results from utilization of sulfide formed by sulfate- and sulfur-reducing microorganisms.

Key words: Lost City hydrothermal field, microbial diversity, sulfate-reducing bacteria, 16S rRNA, *dsrAB, Desulfotomaculum*.

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Discovery of the Lost City active hydrothermal field in December 2000 (30°N, Mid-Atlantic Ridge, Atlantis Massif) is of interest to geologists, geochemists, and microbiologists due to the unusual chemical composition of the hydrothermal fluid and the mineral composition of the hydrothermal chimneys [1]. According to radiocarbon dating, the age of the hydrothermal sediments varies from several decades to 11800 years [2]. Thus, the Lost City field is related to a long-living hydrothermal system.

The Lost City field is located close to the top of the Atlantis Massif at a depth of 750–800 m. The hydrothermal chimneys (from several cm to 60 m high) consist of aragonite, calcite, and brucite. Morphologically, the chimneys vary from thin and spirelike to pillar-shaped. Active chimneys are washed by "shimmering" warm (20–90°C) water, which rises along their walls. Warm water is also seeping out at the flat tops and terraces, often as gryphons [1]. At the sites of fluid seepage from the surface, carbonate chimneys are covered with filamentous microbial mats; white jellylike products containing microorganisms and carbonates form a nonlithified mass near the gryphons [1].

Presently, all the researchers of the Lost City field accept its relationship to the processes of serpentinization of ultrabasic rocks. Two major hypotheses exist concerning the origin of the hydrothermal fluid of the Lost City field. One of them is based on thermodynamic simulation of the processes of serpentinization. The simulation revealed that the fluid formed in the course of serpentinization may provide for all the characteristics of Lost City hydrothermal chimneys via mixing with the near-bottom ocean water. The second hypothesis considers the Lost City fluid as the result of mixing of the hot fluid formed during serpentinization of peridotite and enriched with the gas phase (H_2, CH_4, CO_2, and H_2S), especially with hydrogen, in the course of phase separation, and the cold ambient water of the subsurface biosphere, where microbial processes occur, including active sulfate reduction with hydrogen by thermophilic sulfate-reducing microorganisms [1, 3].

Active sulfate reduction to sulfide is confirmed by an increase (up to 22–30‰) in ^{34}S content of sulfate of the fluid [2]. The OH^- ions formed in the course of sulfate reduction alkalinize the fluid to pH 9.9. Condi-

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Table 1. Primers used in the present work

Primer	Sequence (5'–3')	Source
27F	AGA GTT TGA TCC TGG CTC AG	DeLong (1992)
1492R	GGT TAC CTT GTT ACG ACT T	Weisburg et al. (1991)
DSR1F	AC**S** CAC TGG AAG CAC G	Wagner et al. (1998)
DSR1Fa	ACC CA**Y** TGG AAA CAC G	Loy et al. (2004)
DSR1Fb	GGC CAC TGG AAG CAC G	Loy et al. (2004)
DSR1Fc	ACC CAT TGG AAA CAT G	Zverlov et al. (2005)
DSR1Fd	ACT CAC TGG AAG CAC G	Zverlov et al. (2005)
DSR4R	GTG TAG CAG TTA CCG CA	Wagner et al. (1998)
DSR4Ra	GTG TAA CAG TTT CCA CA	Loy et al. (2004)
DSR4Rb	GTG TAA CAG TTA CCG CA	Loy et al. (2004)
DSR4Rc	GTG TAG CAG TT**K** CCG CA	Loy et al. (2004)
DSR4Rd	GTG TAG CAG TTA CCA CA	Zverlov et al. (2005)
DSR4Re	GTG TAA CAG TTA CCA CA	Zverlov et al. (2005)

tions in the upwelling fluid (temperature from 20 to 90°C, pH 9–9.9) are suitable for thermophilic alkaliphilic sulfate reducers. Such microorganisms have been previously isolated from terrestrial aquatic ecosystems [4]. Their role in subsurface processes may be related to hydrogen utilization as electron donor and energy source. Bicarbonate may act as a carbon source. In order to check this hypothesis, the rate of microbial sulfate reduction was studied in the jellylike microbial aggregates covering the Lost City carbonate chimneys [3]. The rate of sulfate reduction measured with $^{35}SO_4$ was significant, 17000–28000 μg S l^{-1} day^{-1} [1, 3]. Traditional inoculation of selective nutrient media was carried out in parallel to the rate measurement and revealed the presence of sulfate-reducing microorganisms capable of autotrophic growth. A number of heterotrophic sulfate reducers were also found [3]. However, the biodiversity of sulfate-reducing bacteria (SRB) in the samples collected during the 2002 47th cruise of the R/V *Academik Mstislav Keldysh* was not investigated.

It is known from the literature that the fluid and carbonate chimneys of the Lost City field contain organisms similar to sulfur-oxidizing, sulfate-reducing, anaerobic methane-oxidizing, and methanogenic archaea [5]. Their presence was established by phylogenetic analysis and terminal restriction length polymorphism analysis (T-RFLP) of 16S rRNA genes. The authors did not investigate the microbial communities of jellylike mats described earlier [3]. Moreover, an attempt to amplify the *dsrAB* gene encoding dissimilatory sulfite reductase (the key enzyme and functional marker for sulfate reduction) was unsuccessful [5].

The goal of the present work was a search for the organisms capable of sulfate reduction in microbial aggregates of Lost City carbonate chimneys by molecular cloning. The targets for cloning included both the universal phylogenetic marker (the 16S rRNA gene) and the functional marker for sulfate reduction (the *dsrAB* gene, encoding dissimilatory bisulfite reductase, the key enzyme of sulfate reduction).

MATERIALS AND METHODS

Parts of a hydrothermal chimney containing microbial aggregates were collected from a Mir-2 deep submersible by one of the authors on August 28, 2005 in the 50th voyage of the R/V *Academik Mstislav Keldysh* at the same site (the top of the Poseidon giant chimney, point 12, near the gryphon with water temperature of approx. 50°C, where the 2002 samples were collected (Fig. 1). The sample was hauled on board in a special container with a tight titanium lid and frozen at –20°C.

Part of the sample (2.5 g) was homogenized and used for the isolation of total DNA according to the accepted procedure [6]. The primers used for 16S rRNA gene and *dsrAB* amplification are listed in Table 1. The PCR mixture used for amplification of 16S rRNA genes from genomic DNA contained the following: 5 ml 10× PCR buffer with 200 mM ammonium (Fermentas), 5 μl 2.5 M $MgCl_2$ (Fermentas), 5 μl (0.25 mM) dNTP mixture (Fermentas), 2.5 ml DMSO, 10 pm of each primer (GosNIIgenetika, Russia), 0.25 U thermostable *Taq* polymerase (Fermentas), and 1 μl template DNA (at the concentration exceeding 50 ng). Test tubes with the PCR mixture were placed in a thermocycler block preheated to 95°C to decrease the probability of formation of nonspecific products. Amplification was carried out with a Mastercycler (Eppendorf) according to the following program: initial denaturation, 20 s at 95°C; six denaturation cycles of 10 s at 95°C; primer annealing, 20 s at 45°C; primer elongation, 1.5 min at 72°C; 27 denaturation cycles, 10 s at 95°C; primer annealing, 20 s at

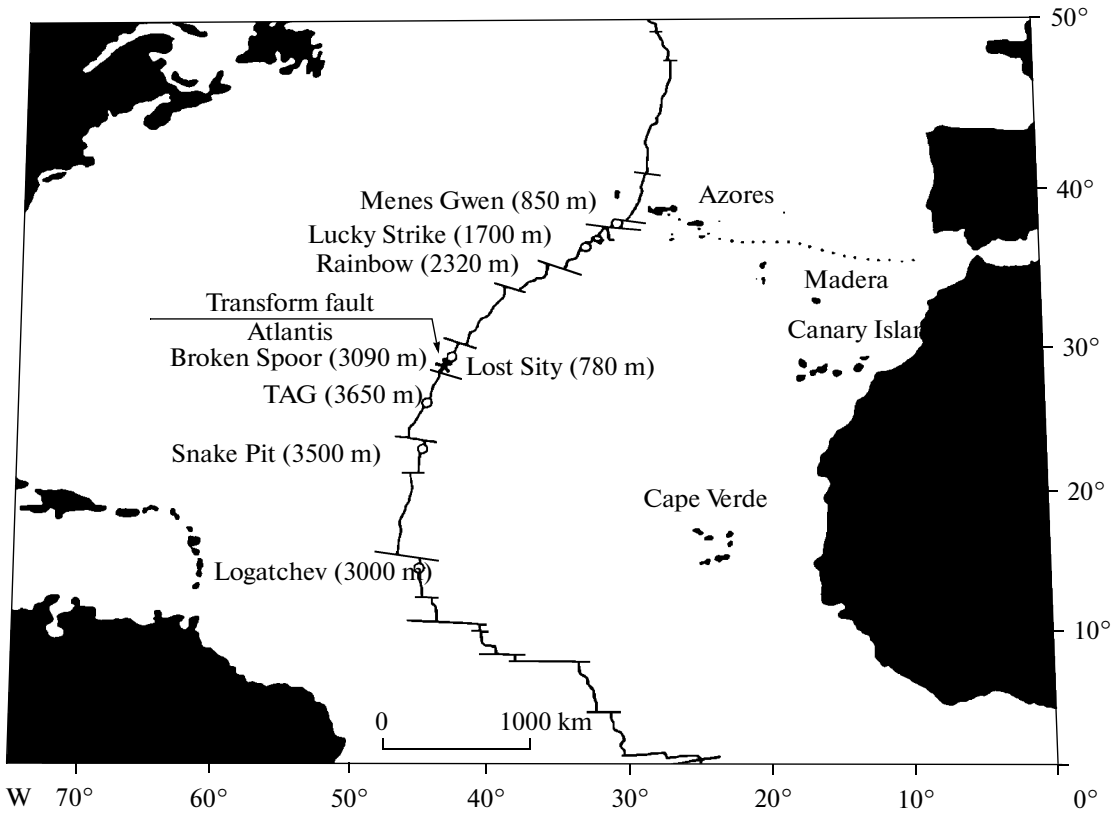

Fig. 1. Location of the Lost City hydrothermal field in the Mid-Atlantic Ridge.

55°C; primer elongation, 1.5 min at 72°C; and final elongation, 3 min at 72°C. PCR amplification of the *dsrAB* genes was carried out with a mixture of degenerated primers dsr1F (a mixture of all the variants of dsr1F forward primers) and dsr4R (a mixture of all reverse dsr4R primers) [7].

For *dsrAB* amplification, the following cycle was empirically determined initial denaturation, 1 min at 95°C, 30 cycles: denaturation, 10 s at 95°C; primer annealing, 20 s at 61°C; elongation, 1 min at 72°C; and final elongation, 10 min at 72°C. PCR products were extracted from 1% agarose gel with the DNA Extraction Kit (Fermentas). PCR products (3 μl of 16S rRNA genes and *dsrAB* genes were cloned into the pTZ57 vector using the InsT/Aclone™ PCR Product cloning Kit (Fermentas) and by electroportation (Easyject Prima, EQUIBIO) were introduced into *E. coli* sp. XL1 cells (GosNIIgenetika). All the stages were carried out according to the manufacturer's recommendations. The transformants were selected on agar LB medium with Xgal/IPTG and ampicillin. White colonies containing the insert were then transferred onto fresh plates. After overnight incubation, the positive clones were collected and lysed by heating at 99°C for 5 min in deionized water. The presence of inserted fragments in the clones was determined by PCR amplification with the primers to the internal site of the M13/pUCF and R vector (Table 1).

Analysis of 16S rRNA and *dsrAB* clone libraries was carried out by restriction fragment length polymorphism (RFLP) method. For this purpose, each PCR product was treated with *Rsa*I and *Hin*6I, *Bsh*l21361 and *Bsu*R1 restriction endonucleases according to the manufacturer's recommendations (Fermentas). After electrophoresis in 2% agarose gel, the clones exhibiting the same restriction profiles were combined into operational taxonomic units (OTU). One clone was randomly chosen from each OTU for sequence analysis. DNA sequences were determined on a Beckman Coulter CEQ 8000 automatic sequencer (GosNIIGenetika, Moscow). For sequences of the *dsrAB* and 16S rRNA genes, M13/pUC and 27F, 1492R primers, respectively, were used.

DNA sequences were analyzed using the BioEdit software package and the GenBank BLAST tool (http://www.ncbi.nlm.nih.gov) [8]. Phylogenetic analysis was carried out using the ARB package (http://www.arb-home.de). The sequences were aligned relative to the sites with a known secondary structure using the FastAlign tool of the ARB package; all alignments were checked manually. The original phylogenetic tree was constructed by the neighbor-joining method for 16S rRNA gene sequences close to completion. Shorter fragments were then added to the tree using the ARB parsimony analysis tool.

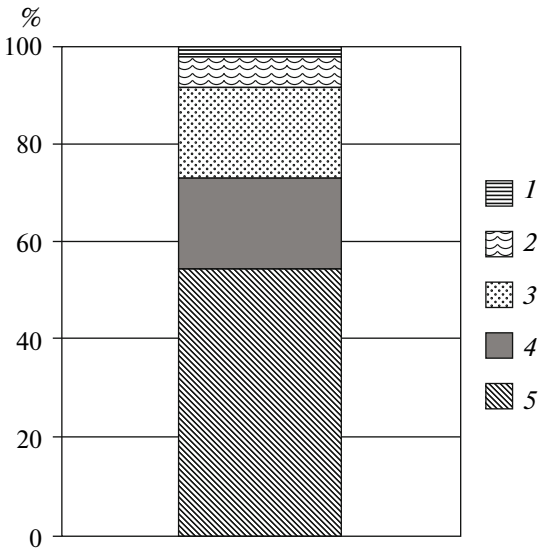

Fig. 2. Microbial community structure based on 16S rRNA gene analysis: Candidate division OD1 (*1*), *Alphaproteobacteria* (*2*), *Epsilonproteobacteria* (*3*), *Firmicutes* (*4*), and *Gammaproteobacteria* (*5*).

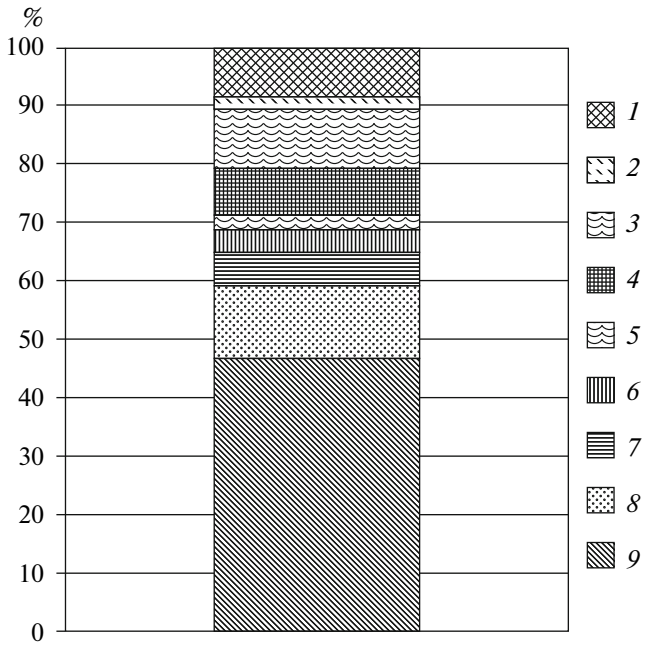

Fig. 3. Species composition of the microbial community based on 16S rRNA gene analysis. Most of the sequences are represented by uncultured bacterial clones. The most closely related forms for which species affiliation is known are presented: unknown *Bacteria* (*1*), *Geosporobacter* (*2*), *Anaerovirgula* (*3*), *Desulfotomaculum* (*4*), *Vibrio* (*5*), *Alteromonas* (*6*), *Marinosulfomonas* (*7*), *Sulfospirillum* (*8*), and *Thiomicrospira* (*9*).

The initial and terminal fragments of the *dsrAB* gene sequence of the clone LC clone dsr_14 were added to an ARB alignment that contained all *dsrAB* sequences of cultured microorganisms and many *dsrAB* clone sequences [9–12] available in the GenBank database. Amino acid sequences were aligned manually using the ARB editor [13]. Nucleotide sequences were aligned according to the aligned amino acid sequences. For phylogenetic inference of DsrAB amino acid sequences, insertions, and deletions were removed from the data set by using a mask (indel filter), which left a total of 543 amino acid positions (alpha subunit, 327 positions; beta subunit, 216 positions) for comparative analysis. A distance matrix tree and bootstrap values (for 100 iterations) were calculated using the Phylogeny Inference Package implemented in the ARB software package. The fragments of the dsrAB clone were inserted into the distance matrix tree without changing the overall tree topology using the ARB parsimony interactive option. All DNA sequences were deposited to NCBI GenBank under accession numbers GQ374159–GQ374173 and GQ403965–GQ403966.

RESULTS

Analysis of *Bacteria* biodiversity based on 16S rRNA gene sequences. A library of 100 clones was obtained with the universal primers for the 16S rRNA gene. The clones with identical restriction profiles were grouped into OTU. Comparison of restriction profiles revealed six OTU, each containing at least two identical clones. A number of clones were represented by unique sequences with restriction profiles having no analogues (data not shown). The distribution of OTU and individual clones, as well as their closest relatives, are presented in Table 2. Sequencing of almost complete and fragmentary 16S rRNA genes revealed members of *Gammaproteobacteria*, *Alphaproteobacteria, Epsilonproteobacteria*, *Firmicutes,* and the "Candidate division OD1" group with undefined taxonomic status (Fig. 2).

Most of the clones belonged to the genus *Thiomicrospira* of the class *Gammaproteobacteria* (Fig. 3). *T. crunogena* was the closest validly described relative of 18 clones in OTU1 (93.8% homology). Phylogenetic analysis revealed that the phylotype belonged to a separate clade comprising also the previously obtained sequences from carbonate chimney of the Lost City hydrothermal field (Table 2). Clone LC1149b154 was the closest relative of OTU1 in this clade (98.9% homology) [5]. This clade comprises also the sequences of uncultured bacteria (clone FS274-19B-03) from the subsurface fluid of the Juan de Fuca rift zone hydrothermal field [14]. The single clone LC_Clone64 belonged to another cluster within *Thiomicrospira*. It was most closely related to *T. arctica* (94.3% similarity).

Sequences of two single clones, LC_Clone93 and LC_Clone57, fell into the genus *Alteromonas* within the *Gammaproteobacteria*. *A. marina* was the closest relative of both (99.4 and 98.9% similarity, respec-

Table 2. Sequenced phylotypes from the 16S rRNA clone library

Clone	Phylogenetic group	Closest relative (GenBank accession no.) / closest validly described relative (GenBank accession no.)	Sequence similarity with that of the closest relative/sequence similarity with that of the closest validly described relative	Number of clones in OTU
LC_OTU1	*Gammaproteobacteria*	Uncultured clone from Lost City chimneys LC1149b154 (DQ228563.1)/*Thiomicrospira crunogena* XCL-2 (CP000109.2)	98.9/93.8	18
LC_OTU2	*Epsilonproteobacteria*	Uncultured clone from Lost City chimneys LC1149b130 (DQ228580.1)/*Sulfospirillum arcachonense* DSMZ 9755 (Y11561)	98.2/97.5	6
LC_OTU3	*Epsilonproteobacteria*	Uncultured clone from Lost City chimneys LC1149b104 (DQ228555.1)/–*	98.3/–*	3
LC_OTU4	*Alphaproteobacteria*	Uncultured clone from Lost City chimneys LC1133B-64 (DQ270644.1)/*Marinosulfonomonas methylotropha* (AY772092.1)	99.3/<95	3
LC_OTU5 (Clone_61)	*Firmicutes*	Uncultured clone from the Juan de Fuca rift zone (AY704403.1)/*Anaerovirgula multivorans* SCA (AB201750.1)	98.9/94.0	6
LC_Clone64	*Gammaproteobacteria*	Uncultured clone (EF491415.1)/*Thiomicrospira arctica* (AJ404731.1)	95.9/94.3	1
LC_Clone32	*Gammaproteobacteria*	Uncultured clone from Lost City chimneys (DQ228563.1)/*Thiomicrospira thermophila* (AB166731.1)	98.9/94.1	1
LC_Clone57	*Gammaproteobacteria*	Uncultured clone SSmNB04-51 from the hydrothermal vent water (AB176127.1)/*Alteromonas marina* SW-47 (AF529060.1)	99.4/98.9	1
LC_Clone93	*Gammaproteobacteria*	Uncultured clone SSmNB04-51 from the hydrothermal vent water (AB176024.1)/*Alteromonas macleodii* R-28032 (AM887684.1)	99.8/99.4	1
LC_Clone72	*Gammaproteobacteria*	Uncultured clone WA_08f from corals (EF123487.1)/*Vibrio tubiashii* ATCC 19109T (X74725.1)	99.7/99.0	1
LC_Clone77	Candidate division OD1	Uncultured clone ODP1230B10.08 from gas hydrate sediments (AB177137.1)/–**	74.3/–**	1
LC_Clone73	*Firmicutes*	Uncultured clone LC1149b139 from Lost City chimneys (DQ228571.1)/*Geosporobacter subterrenus* VNs68 (DQ643978.1)	97.4/86.3	1

Table 2. (Contd.)

Clone	Phylogenetic group	Closest relative (GenBank accession no.) / closest validly described relative (GenBank accession no.)	Sequence similarity with that of the closest relative/sequence similarity with that of the closest validly described relative	Number of clones in OTU
LC_Clone59	*Firmicutes*	Uncultured clone LC1537B-22 from Lost City chimneys (DQ270639.1)/*Desulfotomaculum thermocisternum* strain ST90 (NR_025979)	97.7/88.5	1
LC_Clone2	*Firmicutes*	Uncultured clone LC1537B-22 from Lost City chimneys (DQ270639.1)/*Desulfotomaculum halophilum* strain SEBR 3139 (NR_026061.1)	97.5/90.6	1
LC_Clone100	*Firmicutes*	Uncultured clone LC1537B-22 from Lost City chimneys (DQ270639.1)/*Desulfotomaculum thermocisternum* strain ST90 (NR_025979)	98.4/90.7	1

Notes: * Similarity to the closest validly described species is below 80%.
** Validly described relatives are not known.

tively). Another member of this class, LC_Clone72, belonged to the genus *Vibrio* (Fig. 4).

OTU2, the second numerous group of clones after OTU1, contained the sequences related to *Sulfurospirillum*, class *Epsilonproteobacteria*. *S. arcachonense* was the most closely related taxonomically valid species (97.5% homology). However, the clones isolated by Brazelton et al. from Lost City carbonate chimney [5] were the most closely related sequences. The sequences grouped in OTU3 belonged to a separate clade; all its sequences belonged to uncultured forms. Most of the closely related phylotypes originated from different samples collected in the zone of hydrothermal fields. The phylogenetic tree (Fig. 4) demonstrates two clones most closely related to OTU3 and originating from the Lost City carbonate chimney [5] and from the hot fluids of the Logatchev field [15]. Clade OTU3 probably belongs to the *Epsilonproteobacteria*.

Three clones of OTU4 belonged to a separate clade within the class *Alphaproteobacteria*. *Marinosulfomonas methylotropha* (Table 2) is the closest validly described species. The closest relatives were uncultured clones from hydrothermal ecosystems (Fig. 4). No sequences were revealed belonging to the class *Deltaproteobcteria*, comprising most of the presently known sulfate reducers.

In our 16S rRNA gene clone library, the only microorganisms capable of dissimilatory sulfate reduction are the members of a separate cluster of phylotypes related to spore-forming *Desulfotomaculum*. This cluster contains the sequences of clones 2, 59, and 100; their homology to the validly described *Desulfotomaculum* does not exceed 90% (Fig. 5, Table 2). The phylotypes previously obtained by Brazelton et al. from Lost City carbonate chimney [5] also fall within this cluster. Clones LC1537B-22 and our clone LC_Clone59 were the most closely related ones (97.7% similarity). All the clones of this cluster exhibited different restriction profiles and were represented by unique sequences.

Among the *Firmicutes*, the phylotypes grouped into OTU5 due to identical restriction patterns were the most numerous. *Anaerovirgula multivorans* [16] was their closest validly described relative. The unique clone LC_Clone77 occupies a separate position. This sequence was significantly remote from all the sequences available in NCBI GenBank. It was most closely related to the uncultured clone from methane hydrate samples. However, the similarity between these sequences was only 74.3%.

Analysis of diversity of the microorganisms capable of dissimilatory sulfate reduction based on *dsrAB* gene sequences. A library of 20 clones was obtained using the mixture of degenerative primers to *dsrAB*. Restriction analysis of clones 14 and 19 revealed identical profiles; the clones were grouped in OTU7. The remaining clones exhibited unique restriction profiles. Sequencing of the initial and terminal fragments of clone 14 revealed that the OTU7 nucleotide sequence was closely related to the *dsrAB* gene of *Desulfotomaculum halophilum* DSM 11559 (GenBank accession no. AY626024.1). The similarity between the sequences was 73%. Phylogenetic analysis of the fragments of amino acid sequences demonstrated that clone 14 belonged to the group comprising *D. halophilum, D. alkaliphilum*, and *Desulfotomaculum* sp. Lac2 and was the deepest branch within this clade (Fig. 6).

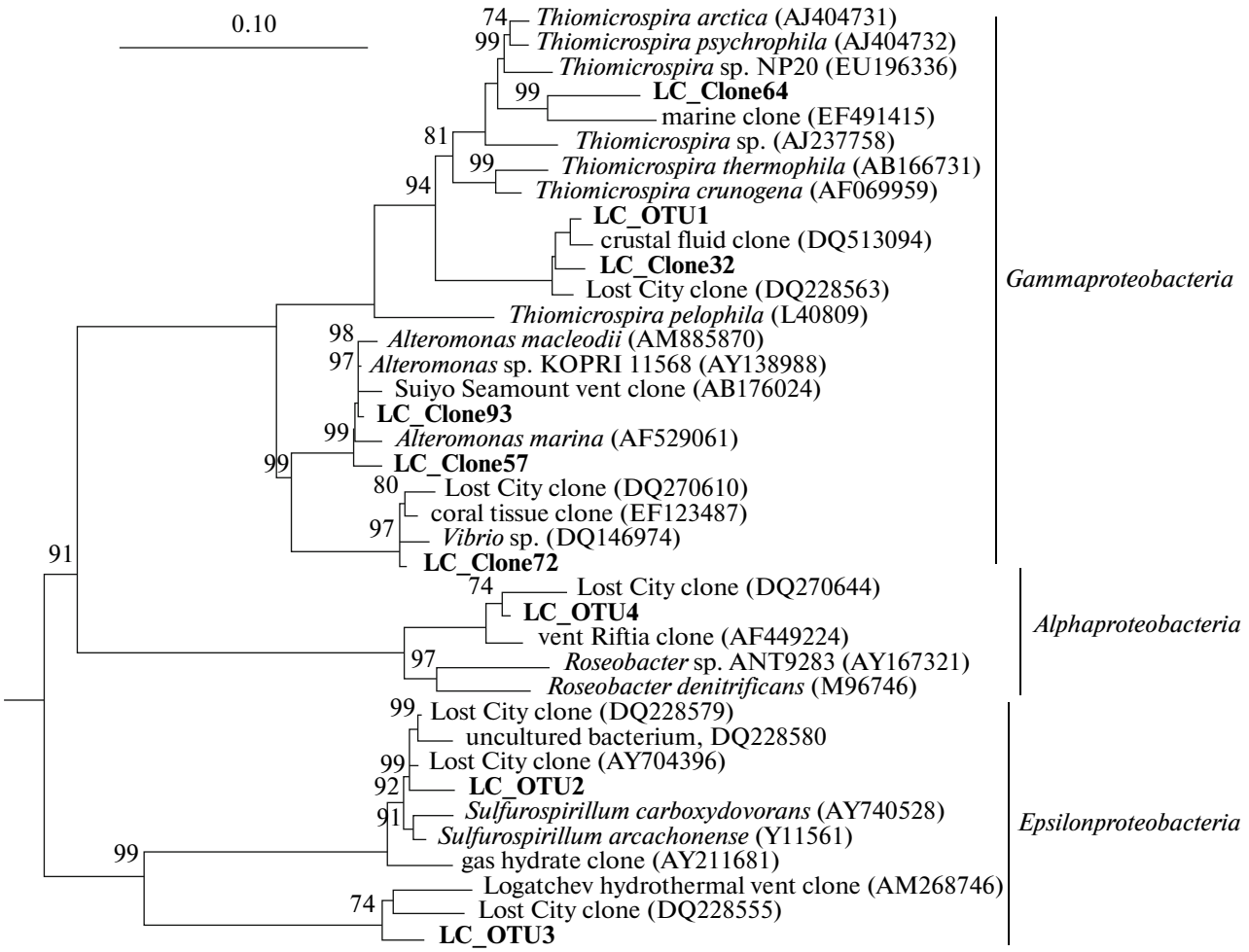

Fig. 4. Phylogenetic position of the *Proteobacteria*-related clones determined on the basis of 16S rRNA gene analysis. The tree was constructed by the neighbor-joining method; the scale shows one replacement per ten nucleotides. *Methanobacterium formicicum* (not shown) was used as an outgroup. The phylotypes obtained in this work are designated in boldface.

The cloned fragments with unique restriction patterns were also sequenced; however, they did not exhibit noticeable similarity to the DNA sequences deposited to GenBank.

DISCUSSION

Diversity of *Bacteria*. Although cloning of 16S rRNA genes from the mat sample was aimed at detection of sulfate-reducing *Bacteria*, it revealed also other members of the microbial community belonging to this domain. Sulfur-oxidizing organisms closely related to *Thiomicrospira* are undoubtedly dominant in the mat sample. These results are in agreement with those of Brazelton et al. [5], who revealed predominance of *Thiomicrospira*-related phylotypes in all samples of the carbonate chimneys and in the fluid with high H_2 content. Direct microscopy of the mat samples from Lost City carbonate chimneys also suggested the presence of the forms probably related to colorless sulfur bacteria with intracellular sulfur inclusions [3]. Thus, molecular analysis of the Lost City mat samples confirmed the conclusion of American researchers concerning predominance of chemolithotrophic sulfur-oxidizing bacterial community, rather than a hydrogen-oxidizing consortium. Similarly to Brazelton et al. [5], we did not detect the members of *Bacteria* traditionally known as hydrogen oxidizers, such as *Deltaproteobacteria* or *Aquificales*. The presence of *Thiomicrospira*-related phylotypes is not surprising, since they are established members of microbial communities of hydrothermal ecosystems. In spite of the unique hydrochemical composition of Lost City fluids, the microbial community forming mats on the chimneys is quite traditional.

Although we did not reveal hydrogen-oxidizing sulfate-reducing *Deltaproteobacteria* in mat samples, the organisms of the OTU2 phylotype are probably capable of elemental sulfur reduction with hydrogen as an electron donor. This phylotype exhibits significant similarity (97.5%) to *Sulfurospirillum arcachonense*. The latter organism, belonging to a separate clade

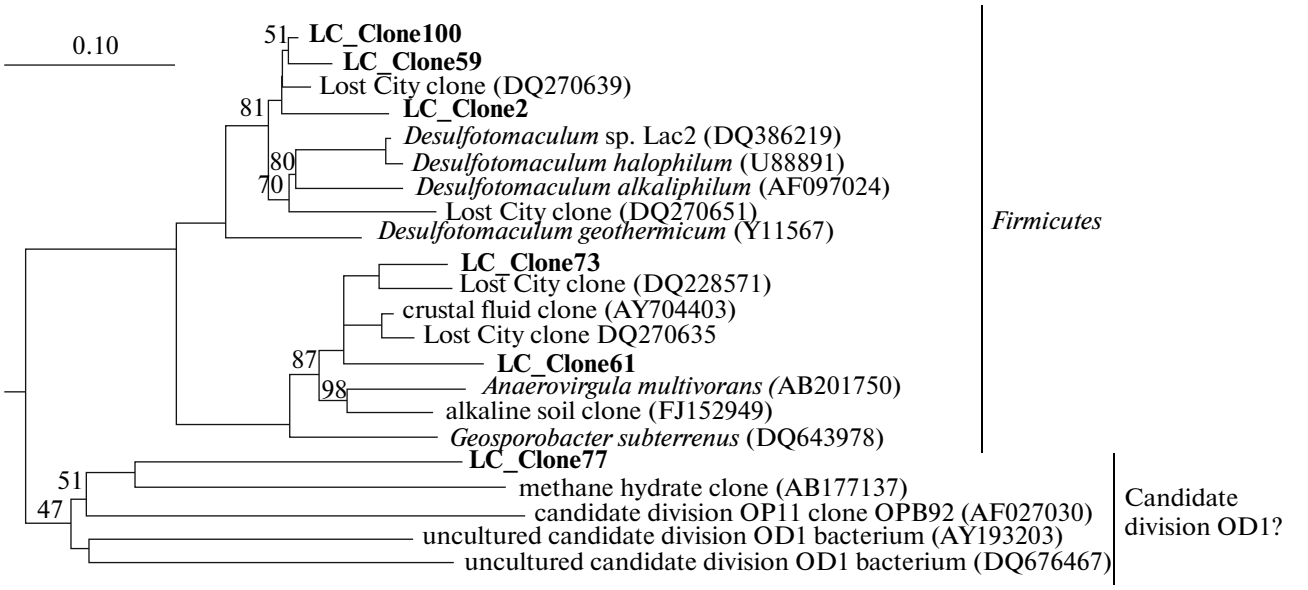

Fig. 5. Phylogenetic position of *Firmicutes* and *Bacteria* of unidentified taxonomic position determined on the basis of 16S rRNA gene analysis. The tree was constructed by the neighbor-joining method; the scale shows one replacement per 10 nucleotides. *Methanobacterium formicicum* (not shown) was used as an outgroup. The phylotypes obtained in this work are designated in boldface.

within *Epsilonproteobacteria*, reduces sulfur with H_2 as an electron donor and is capable of microaerobic growth on hydrogen [17]. All the other described members of this clade (*S. barnesii, S. arsenophilum*, [18], *S. carboxydovorans* [19], and *S. cavolei* [20]) are also able to utilize hydrogen. With hydrogen as an electron donor, *Sulfurospirillum* species require acetate or other organic compounds as carbon sources. In our library, the OTU2 group of clones was the second most numerous after OTU1. Together with the fact that Brazelton et al. [5] revealed a significant number of similar phylotypes in most of the samples from carbonate chimneys and fluids, this may indicate potential importance of *Sulfurospirillum*-related organisms in hydrogen oxidation in the Lost City ecosystem.

A significant part of the clones belonged to spore-forming *Firmicutes*. The qualitatively most significant group of clones, comprised in OTU5, was closely related (with 94% sequence similarity) to the recently described *Anaerovirgula multivorans* [16]. *A. multivorans* is an alkaliphilic chemoorganoheterotroph that obtains energy from fermentation of sugars, organic acids, alcohols, and other compounds.

Since some of the phylotypes revealed in the present work are remote from all the known cultured microorganisms, their possible biogeochemical role is difficult to determine. One of these groups is OTU3, which may be phylogenetically related to *Epsilonproteobacteria*, although sequence homology with all the cultured relatives is significantly below 80%. However, the presence of the phylotypes of this clade not only in Lost City, but also in other hydrothermal fields suggests its probably important role in microbial communities of the hydrotherms. One of the phylotypes (LC_Clone77) had a unique sequence, the only characteristic of which is that it belongs to the domain *Bacteria*. The most closely related sequence available in NCBI GenBank was represented by an uncultured clone and exhibited only 74% similarity.

Sulfate-reducing *Bacteria* in microbial mats of Lost City chimneys. Three unique clones, 2, 59, and 100, were the only phylotypes with a phylogenetic position suggesting their capacity for dissimilatory sulfate reduction. All these clones belonged to one cluster with insignificant variation in 16S rRNA gene sequences; *Desulfotomaculum halophilum, D. alkaliphilum* and *D. thermocisternum* were their closest cultured relatives. However, homology with different *Desulfotomaculum* species did not exceed 90%. Two clones isolated from both Lost City carbonate chimneys and fluids [5] belonged to the same cluster. Brazelton et al. doubted the possibility of sulfate reduction by the organisms of this cluster due to significant divergence of the sequences and the failed attempts to amplify *dsrAB* from the same samples. Our isolation of the *dsrAB* gene from mat samples confirms the possibility of sulfate reduction in microbial mats of Lost City carbonate chimneys. Moreover, since the *dsrAB* gene fragments exhibited the highest homology to the genes of dissimilatory bisulfite reductase from *D. halophilum* and *D. alkaliphilum*, this finding confirms the hypothesis that this group is responsible for sulfate reduction in Lost City carbonate chimneys and fluids.

The results of this work, together with those obtained by Brazelton et al. [5], demonstrate that *Desulfotomaculum*-related phylotypes are probably the

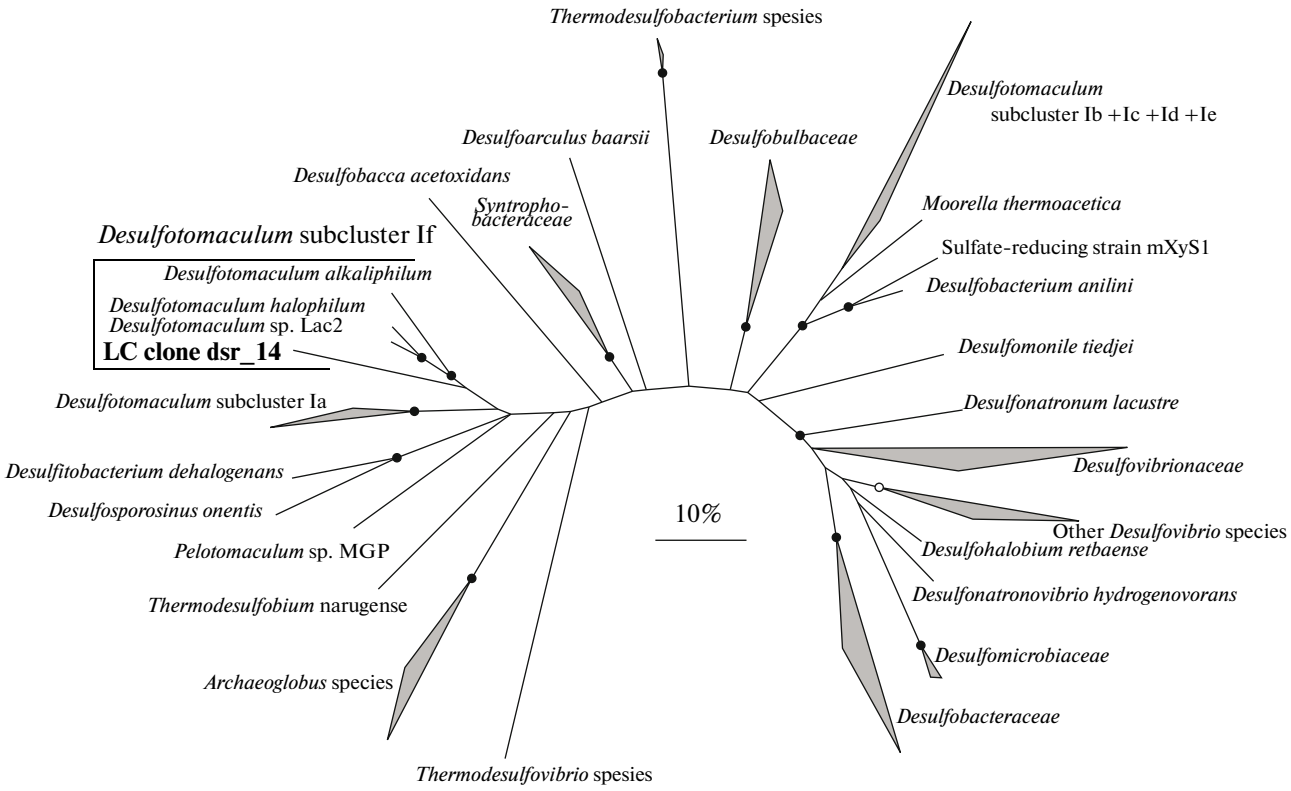

Fig. 6. Phylogenetic tree of DsrAB (FITCH distance matrix, based on amino acid sequences translated from the *dsrAB* nucleotide sequences of over 1750 bp), demonstrating the position of clone 14 from Lost City microbial mats. The partial sequence of LC clone dsr_14 (broken line) was embedded into the tree using the ARB parsimony interactive option of the ARB software package. Scale bar demonstrates 10% sequence divergence. Bootstrap values are represented by black (90%) or white (75 to 90%) circles. For the branches without circles, bootstrap values are below 75%.

only microorganisms carrying out dissimilatory sulfate reduction in microbial mats of Lost City carbonate chimneys. A number of members of this genus, including *D. halophilum* and *D. alkaliphilum*, utilize hydrogen as an electron donor. However, most *Desulfotomaculum* species utilize acetate or other organic compounds as carbon sources for hydrogenotrophic growth. It is therefore probable that hydrogenotrophic sulfate and sulfur reducers are the main group of *Bacteria* utilizing molecular hydrogen in this ecosystem. The primary production is based on utilization of reduced sulfur compounds formed by the hydrogenotrophic microflora. The investigated microbial community is probably a unique interdependent consortium where the energy flow is related to hydrogen of hydrothermal origin, while mass development of the primary producers is based on utilization of sulfide formed by sulfate and sulfur reducers. The investigated microbial communities are the first example of a hydrothermal microbial community where sulfate reduction is carried out exclusively by spore-forming *Desulfotomaculum*. Determination of their advantages in competition with the *Deltaproteobacteria* requires further investigations.

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