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EXPERIMENTAL ARTICLES

Detection and Analysis of Sulfur Metabolism Genes in *Sphaerotilus natans* subsp. *sulfidivorans* Representatives

E. V. Belousova[a], E. Yu. Chernousova[a], G. A. Dubinina[b, 1], T. P. Tourova[b], and M. Yu. Grabovich[a, 1]

[a] *Voronezh State University, Universitetskaya pl. 1, Voronezh, 394893 Russia*

[b] *Winogradsky Institute of Microbiology, Russian Academy of Sciences, pr. 60-letiya Oktyabrya 7, k. 2, Moscow, 117312 Russia*

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Abstract—The lithotrophic capacity of the betaproteobacteria *Sphaerotilus natans* subsp. *sulfidivorans* was confirmed at the genetic level: functional genes of sulfur metabolism were detected (*aprBA*, *soxB*, and *sqr*, coding for adenylyl phosphosulfate reductase, thiosulfate-cleaving enzyme, and sulfide:quinone oxidoreductase, respectively), and the expression of *aprA* and *soxB* genes was demonstrated. An evolutionary scenario for *soxB* genes in *Sphaerotilus* representatives is suggested based on comparative analysis of codon occurrence frequency, DNA base composition (G+C content), and topology of phylogenetic trees. The ancestor bacterium of the *Sphaerotilus–Leptothrix* group was capable of lithotrophic growth in the presence of reduced sulfur compounds. However, in the course of further evolution, the sulfur metabolism genes, including the *soxB* gene, were lost by some *Sphaerotilus* strains. As a result, the lithotrophic *Sphaerotilus–Leptothrix* group split into two phylogenetic lineages: lithotrophic and organotrophic ones.

Keywords: lithotrophy, *Sphaerotilus natans* subsp. *sulfidivorans*, gene expression, evolution

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Sulfide springs of the Northern Caucasus are characterized by a unique set of environmental conditions: the presence of organic substrates, moderately high temperatures (30–40°C), and constant influx of hydrogen sulfide, which promotes the development of sulfur-oxidizing communities of prokaryotes. We were the first to discover that, in the natural moderately thermal sulfide springs in the Northern Caucasus region, the communities of filamentous sulfur-oxidizing bacteria contain as one of the dominant forms representatives of the genus *Sphaerotilus* [1–3], previously considered to be typical heterotrophs, and we described a new subspecies *Sphaerotilus natans* subsp. *sulfidivorans* [4]. Representatives of this new subspecies are able to grow lithotrophically by oxidation of reduced sulfur compounds. In these bacteria, adenylyl phosphosulfate (APS) reductase and sulfite oxidoreductase activities were detected.

However, the understanding of the enzymatic pathways of transformations of reduced sulfur compounds in *S. natans* subsp. *sulfidivorans* representatives is still incomplete. It remains unclear which enzymatic systems participate in the oxidation of thiosulfate, sulfide, and elemental sulfur.

Enzymatic pathways used by prokaryotes for dissimilatory oxidation of sulfur compounds are known to be highly variable [5, 6]. Sulfide oxidation often proceeds through the action of the widespread enzyme, sulfide:quinone oxidoreductase (SQR) encoded by the *sqr* gene [7]. Experimental data have accumulated demonstrating that reverse dissimilatory sulfite oxidoreductase (rDsr) plays an important role in the processes of sulfide or elemental sulfur oxidation to sulfite [8]. Further oxidation of sulfite to sulfate may be mediated by two different enzymatic systems. Functioning of the first system is not coupled to electron transport chain (ETC), and ATP is synthesized via substrate-level phosphorylation: APS reductase (*aprBA* genes) catalyzes sulfite binding to AMP, which results in the formation of APS that is transformed to sulfate under the effect of either ATP sulfurylase (Sat) or APS:phosphate adenylyltransferase (APAT). Functioning of the second system is coupled to ETC, and ATP is synthesized through oxidative phosphorylation: sulfite is oxidized by a sulfite:acceptor oxidoreductase, which catalyzes AMP-independent oxidation of sulfite to sulfate [5, 9].

In addition to sulfide and sulfur, many lithotrophic sulfur-oxidizing prokaryotes are able to use thiosulfate as an electron donor [5, 10, 11]. Several different enzymatic systems and pathways can be distinguished that are involved in dissimilatory oxidation of thiosulfate [5, 6, 10]; in particular, these are (1) thiosulfate oxidation through polythionate by thiosulfate dehydrogenase and tetrathionate hydrolase [10] and (2) oxidation to sulfate under the effect of the Sox periplasmic enzymatic complex, with or without sulfur globule formation.

[1] Corresponding author; e-mail: gdubinina@mail.ru, margarita_grabov@mail.ru

Primers used in this work

Primer name	5'–3' sequence	Specificity
AprB-1-FW	TGC GTG TAY ATH TGY CC	*aprBA* [7]
AprA-5-RV	GCG CCA ACY GGR CCR TA	
AprA-1-FW	TGG CAG ATC ATG ATY MAY GG	
693F	ATC GGN CAR GCN TTY CCN TA	*soxB* [6]
1446B	CAT GTC NCC NCC RTG YTG	
1164F	AAR TTN CCN CGN CGR TA	
432F	GAY GGN GGN GAY ACN TGG	
G3-199F	TBT AYS AGC CGG GWC TKC TBT	*sqr* [8]
G3-566R	GGY GCM ACS GGG CAT TTG	

The pathways of sulfide and thiosulfate transformations used by *S. natans* subsp. *sulfidivorans* for lithotrophic growth are still unclear. Since biochemical methods of evaluating the activity of the enzymes involved in these processes present certain problems; the determination of genes encoding relevant enzymes and of levels of their expression is an appropriate way to clarify the issue.

Therefore, the aim of the present work was to reveal genes coding for the enzymes of sulfur metabolism in *S. natans* subsp. *sulfidivorans* strains capable of lithotrophic growth, and to determine the expression levels of these genes under aerobic and microaerobic conditions of growth.

MATERIALS AND METHODS

Strains and cultivation techniques. This work used *S. natans* subsp. *sulfidivorans* strains D-501 and D-507, isolated from the microbial communities of sulfur-oxidizing bacteria of the moderately thermal sulfide spring Petushok (Goryachii Klyuch, Krasnodar krai). Bacteria were cultured under aerobic and microaerobic conditions [4].

Isolation of total cell RNA fraction. Total cell RNA was isolated as described in [12]. The quality of RNA was controlled by electrophoresis in denaturing polyacrylamide gel (4% PAAG supplemented with 8 M urea). Concentration of the preparations was determined on an ND-1000 spectrophotometer.

Generation of cDNA copies (reverse transcription). Reverse transcription was performed according to the manufacturer's (Fermentas) protocol. A mixture of RNA (2 μg) with relevant primer (4 pmol) was melted for 10 min at 68°C; then, master mix was added, containing revertase buffer (250 mM Tris-HCl, pH 8.3, 250 mM KCl, 20 mM $MgCl_2$, and 50 mM DTT), dNTP (0.2 mM final concentration in a sample), and ribonuclease inhibitor. The tubes were rapidly cooled on ice, 40 units of reverse transcriptase MmulV (Revert Aid™) were added, and the reaction was allowed to proceed at 42°C for 40 min. For inactivation of the enzyme, samples were heated at 85°C for 5 min.

Polymerase chain reaction. To reveal the *soxB, aprBA*, and *sqr* genes, degenerate primers listed in the table were used. PCR was performed in 10 μL of a mixture containing 0.6 μL of 10 pM primer, 1.2 μL (~50 ng) of purified PCR product, 2.2 μL of deionized water, and 6 μL of standard CEQ Dye Terminator Cycle Sequencing kit (Beckman Coulter, United States). Amplification of the gene fragments was performed according to [13–15].

Quantitative PCR of the *soxB* and *aprBA* gene fragments was performed on a DT-322 device (DNK-Tekhnologiya, Russia) using a SYBR Green 1 (Invitrogen, United States) dye intercalating double-stranded DNA. The following program was used for cDNA amplification: 1 min at 94°C, for preliminary melting of cDNA and primers; then, 40 cycles of 20 s at 94°C (DNA denaturation), 23 s at 48–52°C (primer annealing), and 50 s at 72°C (DNA fragment synthesis). The temperature of annealing was selected individually for each pair of primers. In the case of *aprBA* genes, the touch-down PCR technology was used: over the first 8–10 cycles, the annealing temperature was decreased gradually from the maximum acceptable for the particular primer pair (60°C) to a

value 5–6°C below the maximum, and the rest of the cycles were performed according to a standard program. The fluorescent signal was evaluated at the end of each cycle for 15 s. Experiments were performed with two independent batches of RNA in triplicates. In all cases, the quality of PCR products was evaluated by electrophoresis in 5% PAAG (210 V, 100 mA). Standard Quick-Load™ 100bp DNA Ladder (New England Biolabs) set was used as molecular weight markers. Quantitative analysis of the level of gene expression was performed with the use of the q_PCR (DNK-Tekhnologiya) software. Relative quantity of synthesized amplicons was calculated according to the formula: $2^{\Delta Ct}$ [16].

Sequencing of gene fragments was performed on an CEQ2000 XL (Beckman Coulter) automated sequencer according to the protocol proposed by the VNTK Gene activity, Institute of Biochemistry and Physiology of Microorganisms, Russian Academy of Sciences.

Primary analysis of the obtained nucleotide sequences was performed with BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi), ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/), and TCoffe (http://tcoffe.crg.cat/) software packages.

Phylogenetic analysis. To find out whether *soxB* gene is subject to horizontal transfer in the *Sphaerotilus-Leucothrix* group, nucleotide sequences of 16S rRNA and *soxB* genes were compared with the corresponding sequences available in the RDPII (http://rdp.cme.msu.edu) and NCBI (http://www.ncbi.nlm.nih.gov) databases.

Sequences of 16S rRNA genes were aligned using the CLUSTALW software [17]. Phylogenetic dendrograms were constructed using the Treecon software package [18]. The statistical significance of branching points in dendrograms was evaluated by bootstrap analysis of 1000 alternative trees. Individual codon occurrence frequencies were calculated and correspondence analysis was performed using the Codon W software package (http://codonw.sourceforge.net/). Content of G+C pairs in the DNA of *soxB* genes was calculated using the jPhydit software (http://plaza.snu.ac.kr/~jchun/jphydit/).

Deposition of nucleotide sequences. Nucleotide sequences of *soxB, aprA*, and *sqr* genes have been deposited in the GenBank database under accession numbers HQ696789, JF510498, and JN570725, respectively.

RESULTS AND DISCUSSION

Strains D-501^T and D-507 were used as representative strains of *S. natans* subsp. *sulfidivorans* in the analysis of genes of sulfur metabolism enzymes. The strains differed in their metabolism: D-507 was capable of lithotrophic growth independently of oxygenation conditions, while in D-501^T lithotrophic growth occurred only under microaerobic conditions, at an oxygen concentration of 2 mg/L.

Detection of sulfur metabolism genes *soxB, aprBA*, and *sqr*. To detect the *soxB* gene, coding for SoxB hydrolase of the thiosulfate oxidizing complex Sox, we used degenerate primers 693F and 1164B, designed on the basis of comparative analysis of nucleotide sequences [6]. Reverse transcription with further PCR analysis demonstrated the expression of *soxB* gene in cells of *S. natans* subsp. *sulfidivorans* strains D-501^T and D-507. Amplification yielded a fragment of expected length (471 bp), with a rather high sequence identity (up to 91%) to those of bacterial *soxB* genes available in the NCBI database. These results agree completely with biochemical data on the specific features of sulfur metabolism in the bacteria under study. The multienzyme Sox complex catalyzes complete transformation of thiosulfate to sulfate with or without formation of sulfur globules as intermediate products. Earlier, activity of the multienzyme Sox complex was observed in cells of the sulfur-oxidizing *S. natans* subsp. *sulfidivorans* strains under study [3].

For the detection of *aprBA* genes, coding for the dissimilatory adenosine 5'-phosphosulfate reductase, we used primers AprB1Fw and AprA5RV, allowing for amplification of the genes of both subunits of the enzyme, and primers AprA1Fw and AprA5RV, allowing for amplification of the subunit A gene [7]. RT-PCR resulted in products of expected length (1300 and 400 bp, respectively) for both *S. natans* subsp. *sulfidivorans* strains D-501^T and D-507. Comparison of nucleotide sequences of the products obtained in PCR with the closest sequences in the NCBI database showed their high identity level with sequences of *aprBA* genes of other organisms (up to 96%). This matched the biochemical results obtained for strains D-501^T and D-507 previously: they exhibited high activity of adenosine 5'-phosphosulfate reductase, which performs substrate-level phosphorylation in the course of oxidation of sulfite to sulfate [3].

To reveal the *sqr* gene coding for the sulfide:quinone oxidoreductase, primers G3-199F and G3-566R [8] were used. Both for *S. natans* subsp. *sulfidivorans* strain D-501^T and D-507, amplification resulted in a PCR product of the expected length (~310 bp). However, in addition to this product, nonspecific products of amplification were also present. In this connection, we did not further evaluate the expression of this gene under aerobic and microaerobic conditions. Comparison of the sequenced specific PCR product with the closest sequences from the NCBI database showed its high identity level (up to 82%) with *sqr* gene sequences of various sulfur-oxidizing prokaryotes.

Expression of *soxB* and *aprA* genes. For quantitative evaluation of the expression level of the *soxB* and *aprA* genes under microaerobic and aerobic conditions, quantitative PCR was performed. It was shown

that under microaerobic conditions, *soxB* gene expression in strain D-501^T cells increased 7.23 ± 1.27 times, and the expression of the *aprA* gene increased 3.78 ± 0.64 times. These data demonstrate the induction of lithotrophy in strain D-501^T cells at an oxygen content of about 2 mg/L in the liquid medium (under microaerobic conditions); this apparently allows efficient adaptation to changes in oxygenation regime in natural springs [3]. Sensitivity of the enzymes of oxidative sulfur metabolism to oxygen may be the physiological explanation of the activation of lithotrophic metabolism in D-501^T under microaerobic conditions.

Hypothetical scenario of *soxB* gene evolution in representatives of the *Sphaerotilus–Leptothrix* group. Until now, all of the studied representatives of the genus *Sphaerotilus* have been known as components of iron-containing ecosystems and were referred to the group of organoheterotrophic iron bacteria. The ability to oxidize reduced sulfur compounds is a feature atypical of the genus representatives. Therefore, it was of interest to determine whether the ability of *S. natans* subsp. *sulfidivorans* strains to oxidize sulfur compounds is a consequence of horizontal gene transfer.

Horizontal gene transfer can be revealed by a number of characteristic features: (1) major non-conformities between gene's and species phylogeny; (2) difference in the nucleotide composition (G+C content) of a gene from the rest of the genome, suggestive of the alien origin of this gene; (3) difference in the occurrence frequency of certain codons in a gene. Alien origin of a gene may also be manifested through its high similarity level with a gene from a distant taxon in the absence of a similar gene in phylogenetically close relatives.

The results of phylogenetic analysis of *soxB* gene sequences for strain D-507 correlate with strain's phylogeny according to 16S rRNA gene sequence analysis. In the trees of 16S rRNA and *soxB* genes, strain D-507 forms a common cluster with *Leptothrix cholodnii* SP-6 with the high bootstrap values of 97 and 93%, respectively (Figs. 1 and 2). The identity levels between nucleotide sequences of *soxB* and 16S rRNA genes and the corresponding genes from the closest relative *L. cholodnii* SP-6 were 90.7 and 96.4%, respectively.

DNA base compositions of D-507 *soxB* gene and of the total genome DNA of this strain were fairly close, 67.1 and 69.2 mol % G+C, respectively. Similarly, in *L. cholodnii* SP-6, the molar fraction of guanine plus cytosine in the *soxB* gene and in the total genome DNA were close, 66.8 and 69.6 mol % G+C, respectively. The genomic content of guanine and cytosine pairs is a constant characteristic of an organism, therefore, a more than 5% difference in the G+C content between a particular gene and the whole genome may evidence gene acquisition via a horizontal transfer event. In the strains under consideration, the differences in the G+C contents between *soxB* genes and the whole genomes did not exceed 3%; therefore, there were no indications of the alien origin of the *soxB* genes in strains D-507 and *L. cholodnii* SP-6. At the same time, the DNA base compositions of *soxB* genes in strains D-507 and *L. cholodnii* SP-6 are practically the same.

According to the results of our analysis of codon occurrence frequency in *soxB* genes, strain *L. cholodnii* SP-6 also turned out to be the closest to strain D-507. The frequencies of codon occurrence in *soxB* genes in these two strains were practically identical.

Previously, a hypothesis of the evolution of sulfur-oxidizing prokaryotes based on phylogenetic analysis of *soxB* genes has been proposed [6].

The results of our study allow us to expand this hypothesis by proposing a scheme of evolution of *soxB* genes in sulfur-oxidizing representatives of the genus *Sphaerotilus*: *S. natans* subsp. *sulfidivorans*. Filamentous iron bacteria of the genera *Leptothrix* and *Sphaerotilus* are very close by many properties and therefore are traditionally considered as a single *Sphaerotilus–Leptothrix* group. Strain *L. cholodnii* SP-6, as many other representatives of this group, is a chemoorganotroph able to oxidize iron and/or manganese and to deposit their oxides in the sheaths covering the filaments. Iron and manganese oxidation and deposition of the oxides in sheath are not coupled to energy conservation. Reduced iron and manganese species are used to remove a toxic product of cell metabolism, H_2O_2. However, the ability to oxidize sulfur has never been detected in *L. cholodnii* SP-6, as in other representatives of the genus *Leptothrix*.

The similarity of *soxB* gene nucleotide sequences and their practically identical G+C contents and codon occurrence frequencies in *S. natans* subsp. *sulfidivorans* D-507 and *L. cholodnii* SP-6 allows the assumption to be made that there was a common ancestor for these organisms. We do not consider the possibility of exchange of sulfur metabolism genes between the two species, as well as adoption of *soxB* genes from other contemporary species through horizontal transfer, because the above results on the DNA base compositions provide no evidence that the analyzed *soxB* genes are alien to the genomes of *S. natans* subsp. *sulfidivorans* D-507 and *L. cholodnii* SP-6.

Most probably, the ancestor bacterium possessed the capacity for lithotrophic growth in the presence of reduced sulfur compounds. This is evidenced by the following facts: first, oxidation of reduced sulfur compounds by strain *S. natans* subsp. *sulfidivorans* D-507 is coupled to energy metabolism, which has been confirmed at biochemical and genetic levels; second, in the genome of *L. cholodnii* SP-6, *soxB* and *aprBA* genes have been detected (genome project *"Leptothrix cholodnii* SP-6," http://www.ncbi.nlm.nih.gov/nuccore/CP001013.1). At the same time, the absence of experimental proof of the ability of *L. cholodnii* SP-6

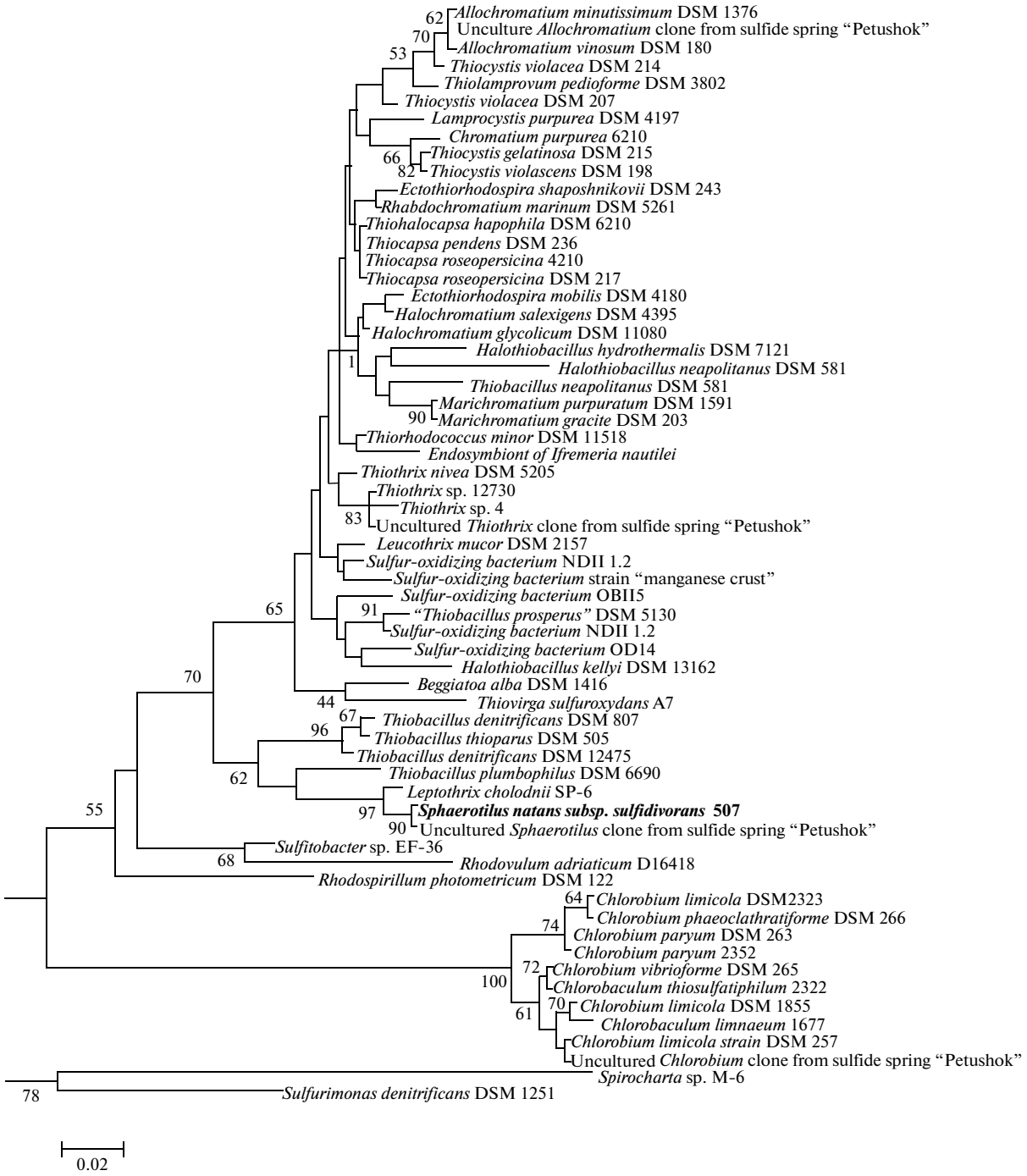

Fig. 1. Phylogenetic tree constructed using the neighbor-joining method through comparison of 16S rRNA gene nucleotide sequences. The scale bar indicates the evolutionary distance corresponding to 2 substitutions per each 1000 nucleotides. The numerals indicate the significance of the branching order determined by bootstrap analysis of 1000 alternative trees (only values over 50% are shown).

to oxidize sulfur compounds may be explained by (a) necessity for selection of special conditions for its lithotrophic growth or (b) lack of other sulfur metabolism genes (*sqr* and *rDsr*) in its genome (http://www.ncbi.nlm.nih.gov/nuccore/CP001013.1).

The question as to the functional importance and expression activity of *soxB* gene in *L. cholodnii* SP-6 remains open. There are no data on the ability to oxidize sulfur compounds and the presence of dissimilatory sulfur metabolism genes in other representatives

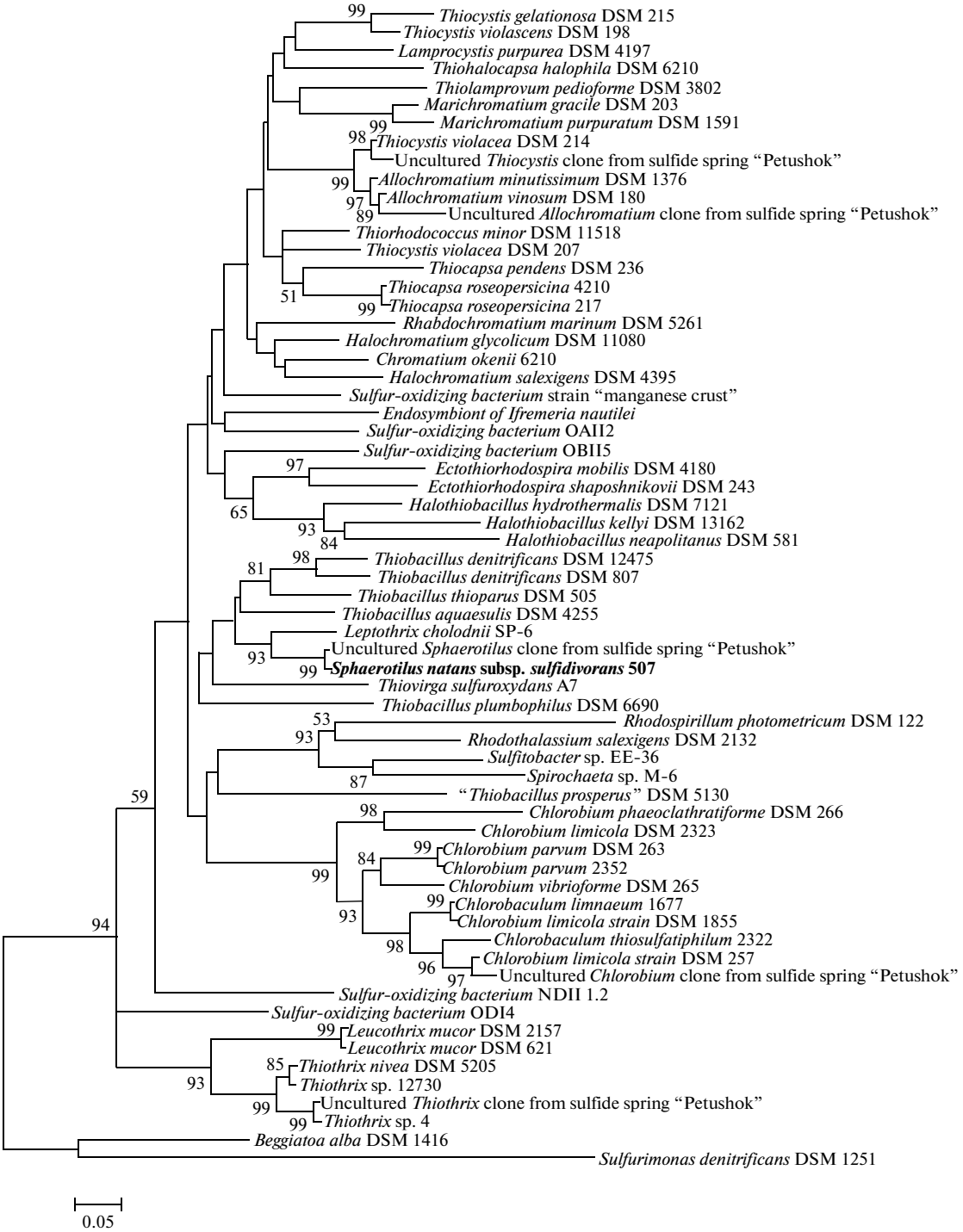

Fig. 2. Phylogenetic tree constructed using the neighbor-joining method through comparison of nucleotide sequences of genes coding for the SoxB component of the thiosulfate-oxidizing complex. The scale bar indicates the evolutionary distance corresponding to 5 substitutions per each 100 amino acids. The numerals indicate the significance of the branching order determined by bootstrap analysis of 1000 alternative trees (only values over 50% are shown).

of the genus *Leptothrix* (*L. mobilis, L. discophora, L. lopholea, L. cholodnii* LMG7171); however, no studies have been performed in this direction.

Thus, the presented data prove, at the genomic level, the capacity for lithotrophy in representatives of *S. natans* subsp. *sulfidivorans*. Analysis of the functional genes determining the capacity of *S. natans* subsp. *sulfidivorans* representatives for lithotrophic growth in the presence of reduced sulfur compounds allowed us to establish the key role of such genes as

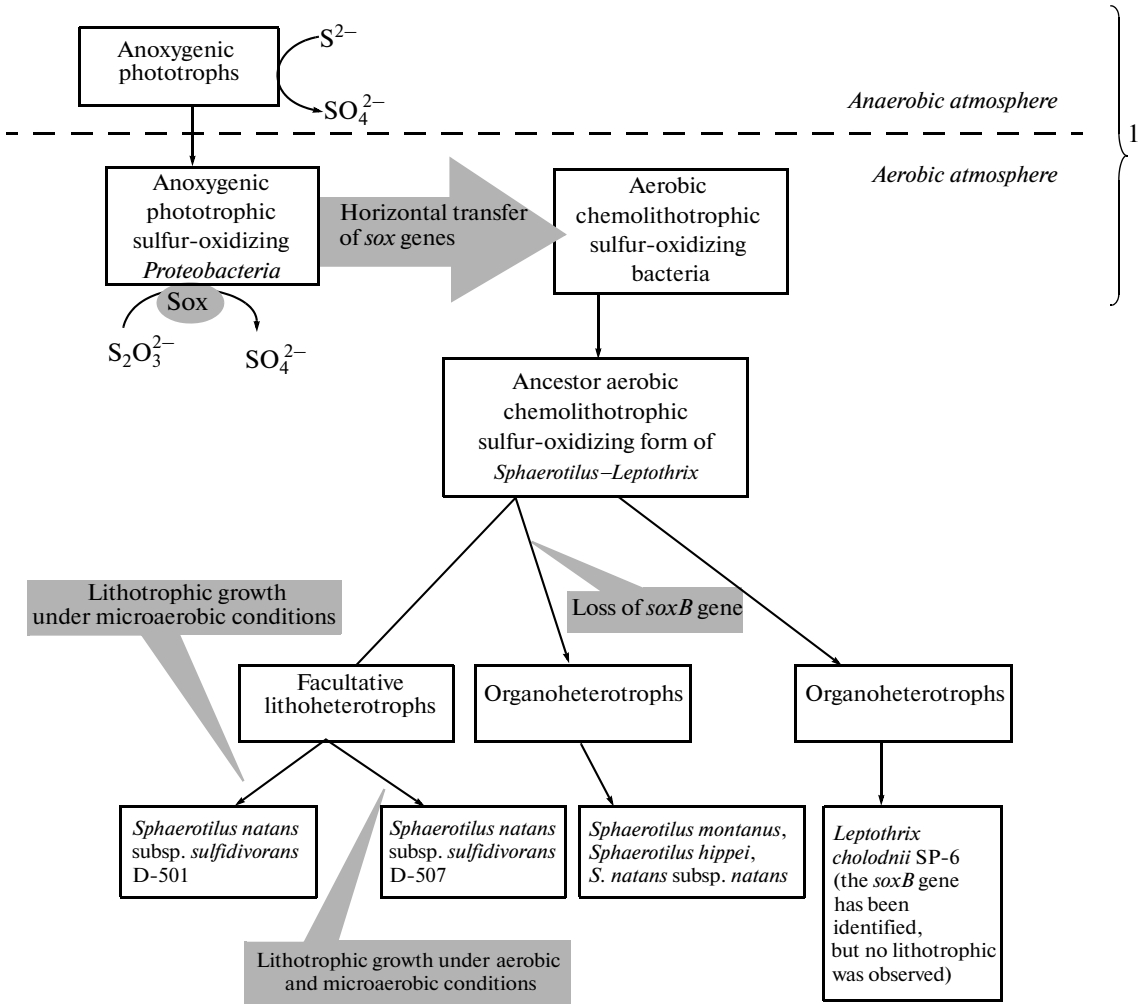

Fig. 3. Hypothetical scenario of the *soxB* gene evolution in the *Sphaerotilus–Leptothrix* group representatives. 1, data adopted form [6].

aprBA, soxB, and *sqr*, coding for APS reductase, thiosulfate-cleaving complex, and sulfide:quinone oxidoreductase. We have shown a considerable increase in the expression of *aprBA* and *soxB* genes under microaerobic conditions compared to aerobic ones. The data of genetic studies correlate with biochemical data: in representatives of *S. natans* subsp. *sulfidivorans*, APS reductase, sulfite oxidoreductase, and Sox complex activities have been detected.

In Fig. 3, we present a hypothetical schematic scenario of *soxB* gene evolution in representatives of the *Sphaerotilus–Leptothrix* group.

We have not found *soxB* genes in representatives of *S. montanus, S. hippei*, and *S. natans* subsp. *natans*. Probably, in the process of evolution, genes of sulfur metabolism, *soxB* gene in particular, were lost in most of *Sphaerotilus* strains, which may be connected with the adaptation of these bacteria to living in iron-containing ecosystems. This loss could be a result of evolutionary selection of optimal physiological mode of existence. As a result, the lithotrophic *Sphaerotilus–Leptothrix* group divided into two physiological lineages: lithotrophic and organotrophic bacteria.

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REFERENCES

1. 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, no. 2, pp. 219–223.
2. Chernousova, E.Yu., Akimov, V.N., Gridneva, E.V., Dubinina, G.A., and Grabovich, M.Yu., Biodiversity and monitoring of colorless filamentous bacteria in sulfide aquatic systems of North Caucasus region, *Microbiology* (Moscow), 2010, vol. 79, no. 5, pp. 672–678.

3. Gridneva, E.V., Grabovich, M.Yu., Dubinina, G.A., Chernousova, E.Yu., and Akimov, V.N., Ecophysiology of lithotrophic sulfur-oxidizing *Sphaerotilus* species from sulfide springs in the Northern Caucasus, *Microbiology* (Moscow), 2009, vol. 78, no. 1, pp. 76–83.

4. Gridneva, E., Chernousova, E., Dubinina, G., Akimov, V., Kuever, J., Detkova, E., and Grabovich, M., Taxonomic investigation of representatives of the genus *Sphaerotilus:* descriptions of *Sphaerotilus montanus* sp. nov., *Sphaerotilus hippei* sp. nov., *Sphaerotilus natans* subsp. *natans* subsp. nov. and *Sphaerotilus natans* subsp. *sulfidivorans* subsp. nov., and an emended description of the genus *Sphaerotilus, Int. J. Syst. Evol. Microbiol.*, 2011, vol. 61, pp. 916–925.

5. Brusser, T., Lens, P.N.L., and Truper, H.G., The biological sulfur cycle, in *Environmental Technologies to Teat Sulfur Pollution*, Lens, P.N.L. and Pol, L.H., Eds., London, UK: IWA Publishing, 2000, pp. 47–86.

6. Friedrich, C.G., Rother, D., and Bardischewsky, D., Oxidation of reduced inorganic sulfur compounds by bacteria: emergence of a common mechanism?, *Appl. Environ. Microbiol.*, 2001, vol. 67, pp. 2873–2882.

7. Griesbeck, C., Hauska, G., and Schutz, M., Biological sulfide oxidation: sulfide-quinone reductase (SQR), the primary reaction, in *Recent Research Developments in Microbiology*, Pandalai S.G., Ed., 2000, vol. 4, pp. 179–203.

8. Dahl, C., Engels, S., and Pott-Sperling, A.S., Novel genes of the *dsr* gene cluster and evidence for close interaction of Dsr proteins during sulfur oxidation in the phototrophic sulfur bacterium *Allochromatium vinosum, J. Bacteriol.*, 2005, vol. 187, pp. 1392–1404.

9. Kappler, U. and Dahl, C., Enzymology and molecular biology of prokaryotic sulfite oxidation, *FEMS Microbiol. Lett.*, 2001, vol. 203, pp. 1–9.

10. Kelly, D.P., Shergill, J.K., Lu, W.P., and Wood, A.P., Oxidative metabolism of inorganic sulfur compounds by bacteria, *Antonie van Leeuwenhoek*, 1997, vol. 71, pp. 95–107.

11. Kletzin, A., Metabolism of inorganic sulfur compounds in Archaea, in *Archaea. Evolution, Physiology, and Molecular Biology*, Garrett, R.A., and Klenk, H.-P., Eds., Oxford: Blackwell, 2006, pp. 261–274.

12. Favre-Bonte, S., Joly, B., and Forestier, C., Consequences of reduction of *Klebsiella pneumonia* capsule expression on interactions of this bacterium with epithelial cells, *Infect. Immun.*, 1999, vol. 67, pp. 554–561.

13. Meyer, B., Imhoff, J.F., and Kuever, J., Molecular analysis of the distribution and phylogeny of the *soxB* gene among sulfur-oxidizing bacteria—evolution of the Sox sulfur oxidation enzyme system, *Environ. Microbiol.*, 2007, vol. 9, no. 12, pp. 2957–2977.

14. Meyer, B. and Kuever, J., Molecular analysis of the distribution and phylogeny of dissimilatory adenosine-5'-phosphosulfate reductase encoding genes (*aprBA*) among sulfur-oxidizing prokaryotes, *Microbiology* (UK), 2007, vol. 153, pp. 3478–3498.

15. Pham, V.H., Yong, J.-J., Park, S.J., Yoon, D.-N., Chung, W.-H., and Rhee, S.-K., Molecular analysis of the diversity of the sulfide:quinone reductase (sqr) gene in sediment environments, *Microbiology* (UK), 2008, vol. 154, p. 3112–3121.

16. Suvorova, I.A., Tutukina, M.N., Ravcheev, D.A., Rodionov, D.A., Ozoline, O.N., and Gelfand, M.S., Comparative genomic analysis of the hexuronate metabolism genes and their regulation in gammaproteobacteria, *J. Bacteriol.*, 2011, vol. 193, no. 15, pp. 3956–3963.

17. Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F., and Higgins, D.G., The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools, *Nucleic Acids Res.*, 1997, vol. 25, pp. 4876–4882.

18. 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, no. 5, pp. 569–570.

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