Thiothrix caldifontis sp. nov. and *Thiothrix lacustris* sp. nov., gammaproteobacteria isolated from sulfide springs

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Five strains of filamentous, sulfur-oxidizing bacteria were isolated from sulfur mats of different sulfide springs from various regions of the Northern Caucasus, Russia. A phylogenetic analysis based on 16S rRNA gene sequence comparison showed that all of the isolates are affiliated with the filamentous, colourless, sulfur-oxidizing bacteria of the genus *Thiothrix* within the *Gammaproteobacteria* and are closely related to *Thiothrix fructosivorans*. All strains are capable of growing heterotrophically, lithoautotrophically with thiosulfate or sulfide as the sole energy source and mixotrophically. Strains G1^T, G2, P and K2 are able to fix molecular nitrogen, but strain BL^T is not. Randomly amplified polymorphic DNA (RAPD)-PCR analysis was used to assess the level of genetic relationships among the *Thiothrix* isolates. The Nei and Li similarity index revealed high genetic similarity among strains G1^T, G2, P and K2 (above 75 %), indicating that they are closely related. In combination with physiological and morphological data, strains G1^T, G2, P and K2 can be considered as members of the same species. The lowest genetic similarity (approx. 20 %) was reached between strain BL^T and the other isolated *Thiothrix* strains. Strains BL^T and G1^T shared 35 % DNA–DNA relatedness and showed 51 and 53 % relatedness, respectively, to *Thiothrix fructosivorans* ATCC 49749. On the basis of this polyphasic analysis, strains G1^T, G2, P and K2 represent a novel species within the genus *Thiothrix*, for which the name *Thiothrix caldifontis* sp. nov. is proposed, with strain G1^T (=DSM 21228^T =VKM B-2520^T) as the type strain. In addition, strain BL^T represents a second novel species, *Thiothrix lacustris* sp. nov., with strain BL^T (=DSM 21227^T =VKM B-2521^T) as the type strain.

The genus *Thiothrix* (family ‘*Thiothrichaceae*’, class *Gammaproteobacteria*) comprises seven species. On the basis of phylogenetic data, *Thiothrix* species are divided

into two main groups. The *Thiothrix nivea* group, including *T. nivea*, *T. unzii* and *T. fructosivorans*, shows 88.3–92.0 % 16S rRNA gene sequence similarity to the Eikelboom type 021N group, which includes *Thiothrix disciformis*, *T. flexilis* and *T. eikelboomii*. *Thiothrix defluvii* is closely related phylogenetically to *T. flexilis* strains of the Eikelboom type 021N group, with 96.8–97.1 % 16S rRNA gene sequence similarity. However, this species is not a member of Eikelboom type 021N (Aruga *et al.*, 2002). The names ‘*Thiothrix ramosa*’ and ‘*Thiothrix arctophila*’ have not been validly published and strain CT3 has not been assigned to a named species; the status of these organisms is uncertain (Unz & Head, 2005).

Abbreviation: RAPD, randomly amplified polymorphic DNA.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA, *gyrB* and *hsp60* gene sequences of strains G1^T and BL^T are respectively EU642573 and EU642572 (16S rRNA gene), FJ032200 and FJ032199 (*gyrB*) and FJ161077 and FJ161076 (*hsp60*).

RAPD patterns of newly isolated *Thiothrix* strains, 16S rRNA gene, *hsp60* and *gyrB* sequence similarity matrices and DNA–DNA hybridization results are available as supplementary material with the online version of this paper.

During the last two decades, the development of molecular biological tools has led to a significant improvement in the field of classification and identification of micro-organisms. Molecular approaches based not only on analysis of the 16S rRNA gene, but also on several housekeeping genes, including *hsp60*, *gyrB*, *rpoD*, *rpoB* and *dnaJ*, have gained in importance because they allow better discrimination of taxa, especially at the species and strain levels (Yamamoto & Harayama, 1998; Kwok *et al.*, 1999; Blackwood *et al.*, 2000). The functional genes *hsp60* (encoding the 60 kDa chaperonin) and *gyrB* (encoding the subunit B protein of DNA gyrase) can be used as additional phylogenetic markers, being characterized by higher rates of evolution in comparison with the 16S rRNA gene and by universality, being present in each genome as a single copy. For several bacteria, a good correlation between DNA–DNA hybridization data and analysis of *hsp60* and *gyrB* gene sequences has been shown (Hatano & Nishii, 1994; Kwok *et al.*, 1999; Yamamoto *et al.*, 1999; Jian *et al.*, 2001).

In a previous study, we found a mass development of filamentous sulfur bacteria as bacterial mats in sulfide springs of the Northern Caucasus region. According to a phylogenetic *in situ/ex situ* analysis, the bacterial mats were dominated by representatives of the genus *Thiothrix* (Chernousova *et al.*, 2008). In this work, we present the comparative analysis of five novel strains of the genus *Thiothrix* obtained from these mats by means of a polyphasic approach including molecular, chemotaxonomic and phenotypic characterization.

Strains G1^T, G2, P and K2 were isolated from bacterial mats of different sulfide springs located in the northern spurs of the Central Caucasus Ridge, in Krasnodar and Stavropol Krai, Russia. These sulfide springs are formed from outflows of deep sulfidic waters and have a temperature of nearly 40 °C. Bacterial mats containing sulfur inclusions are visible and are abundant on drains of the sulfidic waters and on the surfaces of rocks. Strains G1^T and G2 were isolated from the Petushok spring, strain P was from bacterial mats of Proval lake and strain K2 was from Kabardinskii spring. The bacterial mats from which strains G1^T, G2, P and K2 were isolated developed at 33–40 °C, pH 7.3–7.5 and at a sulfide concentration in the water of 3.8–6.4 mg l⁻¹.

Strain BL^T was isolated from bacterial mats located at a shallow site of a lower lake of the Blue lake system (Kabardino-Balkaria). It is the deepest karstic lake in Russia (365 m) and is located at 809 m above sea level. The temperature at the surface of the lake is constant year-round at 9.3 °C and the sulfide concentration is maintained at 3.1 mg l⁻¹ at pH 7.5.

Isolation of pure cultures was carried out on Armbruster medium (Armbruster, 1969). Strains G1^T, G2, P, K2 and BL^T were incubated at 25 °C for 48 h.

Strains G1^T, G2, K2, P and BL^T were analysed by randomly amplified polymorphic DNA-PCR (RAPD-PCR). The cells

were disrupted by three cycles of freezing to –70 °C and thawing at 50 °C with subsequent heating of the cell suspension at 85 °C for 5 min (Bej *et al.*, 1991). DNA was extracted by the phenol method (Ausubel *et al.*, 1994). Genomic RAPD fingerprints were obtained as described by De Bruijn (1992) using the primer M13 (5'-TTATGT-AAAACGACGGCCAGT-3') with subsequent analysis by electrophoresis in 1.5 % (w/v) agarose gels (Supplementary Fig. S1, available in IJSEM Online). Most of the amplified fragments were between 300 and 2000 bp. Strains G1^T, G2, P and K2 shared similar PCR patterns between 900 and 2000 bp, while strain BL^T differed markedly from the other strains in the presence of five fragments between 500 and 1700 bp. The RAPD fingerprint patterns obtained were converted into a binary data matrix by scoring the presence of a band as 1 and its absence as 0. Bands that were not reproducible were excluded from the analyses. Faint and visually indistinguishable bands were also ignored. The binary matrix was subjected to the FreeTree 0.9.1.50 software (Hampl *et al.*, 2001), using the Nei and Li coefficient (Nei & Li, 1979) to generate a similarity matrix. The lowest similarity (approx. 20 %) was reached between strain BL^T and all the other isolated *Thiothrix* strains. High genetic similarity, above 75 %, was observed between strains G1^T, G2, P and K2.

Cell morphology was observed by using an Olympus CX 4 microscope equipped with a phase-contrast device and by transmission electron microscopy with an accelerating voltage of 60 kV. The cell morphology of all strains was similar. Rod-shaped cells, arranged in chains, were seriate in trichomes within a polysaccharide sheath (Fig. 1c). Gliding gonidia were produced from the apex. At the base, the trichomes formed a holdfast. When organisms were grown in the presence of a reduced inorganic sulfur compound, sulfur globules were deposited within cells (Fig. 1a). The top cells of short filaments sometimes formed pin-like bulges during the stationary growth phase (Fig. 1d). In the early stages of exponential growth, spiral forms of filaments were often observed for strains G1^T, G2, P and K2 (a micrograph for strain G1^T is shown in Fig. 1b).

Physiological and biochemical properties of the strains were determined using standard methods (Gerhardt *et al.*, 1981). Morphological and physiological properties of the strains are compared in Tables 1 and 2. All strains were able to grow heterotrophically, autotrophically with thiosulfate or sulfide and also mixotrophically using a combination of reduced sulfur compounds and lactate. Thiosulfate or sulfide were oxidized to sulfate and element sulfur.

Strains G1^T, G2, P and K2 grew on medium without a nitrogen source and were capable of nitrogen assimilation, but strain BL^T was not. The ability of the strains to fix nitrogen was confirmed by the acetylene reduction assay (Stewart *et al.*, 1968).

On the basis of RAPD analysis, physiological and morphological data, strains G1^T, G2, P and K2 can be considered as members of the same species. Strains G1^T

Fig. 1. Morphology of strains BL^T (a, c) and G1^T (b, d). (a) Rosette with sulfur globules; (b) helical cell shape; (c) mucous cover around cells; (d) modified end cells of trichome. Bars, 10 μ m (a), 20 μ m (b, d) and 1 μ m (c).

(as a representative of the first group) and BL^T were studied in more detail.

The 16S rRNA genes of G1^T and BL^T were amplified using primers 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492r (5'-TACGGYTACCTTGTACGACTT-3') (Lane, 1991; Medlin *et al.*, 1988). The PCR products were sequenced by using a CEQ2000 XL automatic sequencer (Beckman Coulter). The known closest relatives of the new isolates were determined by performing sequence database searches and the sequences of closely related strains were retrieved from the GenBank (NCBI; <http://www.ncbi.nlm.nih.gov>) or Ribosomal Database Project (RDPII; <http://rdp.cme.msu.edu>) libraries. Aligning of 16S rRNA gene sequences was carried out using the CLUSTAL_X software package (Thompson *et al.*, 1997). An evolutionary-distance matrix was calculated using the algorithm of Jukes & Cantor (1969). A phylogenetic tree was constructed using the neighbour-joining method. Bootstrap analysis was based on 1000 resamplings. The TREECON software package was used for phylogenetic analysis (Van de Peer & De Wachter, 1994).

On the basis of the distance matrix, 16S rRNA gene sequence similarities indicated that the closest phylogenetic relatives of strains BL^T and G1^T are two strains of *T. fructosivorans*, strain I (98.8 and 98.7% similarity, respectively) and strain Q^T (98.8 and 98.9%), and the sheathed, filamentous bacterium strain CT3 (99.1 and

99.0% similarity). *Thiothrix* sp. CT3 was isolated from an activated sludge treatment plant in Italy by Rossetti *et al.* (2003). These authors showed that there are several differences between *Thiothrix* sp. CT3 and *T. fructosivorans*, but did not propose a novel species for *Thiothrix* sp. CT3. Strains BL^T and G1^T shared 98.8% sequence similarity (Supplementary Table S1). The 16S rRNA gene sequence similarity between strains BL^T and G1^T and the other two species of the co-called *Thiothrix nivea* group, *T. unzii* and *T. nivea*, was respectively 94.5–94.9 and 94.0–95.1%. A phylogenetic tree based on 16S rRNA gene sequences is shown in Fig. 2.

The type strain of *T. fructosivorans*, strain ATCC 49748^T (=strain Q^T), was replaced in further investigations by ATCC 49749 (=strain I), because the culture of strain ATCC 49748^T obtained from the ATCC did not grow. The high level of sequence similarity between strains I and Q^T (16S rRNA gene, 99.4%; *hsp60*, 99.8%; Supplementary Table S1) and their extremely similar phenotypic properties (Howarth *et al.*, 1999) indicate that the two strains belong to the same species. Additionally, they were isolated from the same activated-sludge plant (Williams & Unz, 1985). Therefore, the use of strain ATCC 49749 instead of the type strain for comparative analysis of *gyrB* genes and for DNA–DNA hybridization studies seems justified. *Thiothrix* sp. CT3 was obtained as strain DSM 12730.

Table 1. Differential characteristics of strains BL^T and G1^T, *Thiothrix* sp. CT3 and *T. fructosivorans* I

Strains: 1, BL^T; 2, G1^T (properties of strains G1^T, G2, P and K2 were highly similar; therefore, only data for strain G1^T are presented); 3, *Thiothrix* sp. CT3 (data from Rossetti *et al.*, 2003); 4, *T. fructosivorans* I (=ATCC 49749) (Howarth *et al.*, 1999). For all strains, starch, gelatin, casein are not hydrolysed; sulfide is not formed from cysteine and thiosulfate, indole is not produced, nitrate and fumarate are not used as electron acceptors, no growth is observed in 3 % NaCl, pigments are not formed when cells are grown on aromatic amino acids and acid is not produced from sugars. All strains are positive for oxidase activity and reduction of nitrate to nitrite. All strains utilize acetate, succinate, lactate and pyruvate and do not utilize benzoate, salicylate, glyoxylate, glycolate; mannitol, sorbitol, glycerol, ethanol, butanol, isobutanol, propanol, inositol; lactose, D-glucose, D-galactose, L-sorbose, D-mannose, trehalose; serine, lysine, tryptophan, histidine, phenylalanine, methionine, tyrosine, proline, ornithine, glutamine, peptone, yeast extract or casein hydrolysate.

Characteristic	1	2	3	4
Cell width (µm)	0.9–2.3	0.9–2.2	0.8–2.0	1.0–1.7
Cell length (µm)	4.4–6.3	3.2–6.5	4.3–6.7	4.9–10.0
pH for growth				
Range	6.2–8.2	7.0–8.6	7.0–8.0	6.7–8.0
Optimum	7.0	8.0	7.6	7.6–8.0
Temperature for growth (°C)				
Range	5–32	7–37	10–30	5–32
Optimum	24	25	20–24	25–27
DNA G + C content (mol%) (T _m)	51.4	52	53.4	51.5
Utilization of carbon sources				
Malate	–	–	–	+
Fumarate	+	+	–	+
Oxalate	+	–	–	+
Oxaloacetate	+	+	–	+
Citrate	+	–	–	–
Isocitrate	+	–	–	+
2-Oxoglutarate	+	–	–	–
Formate	–	–	–	+
Aconitate	+	–	–	–
Malonate	+	–	–	–
L-Arabinose	–	–	–	+
Raffinose	–	–	–	+
Sucrose	–	–	–	+
Maltose	–	–	–	+
D-Fructose	–	–	–	+
D-Xylose	–	–	–	+
L-Rhamnose	–	–	–	+
Glutamate	+	–	–	–
Aspartate	+	+	–	–
Cysteine	+	–	–	–
Cystine	+	–	–	–
Asparagine	+	–	–	–
Leucine	–	+	–	–
Isoleucine	–	+	–	–
Catalase activity	–	–	–	+
Assimilation of nitrogen	–	+	–	–

The *hsp60* gene was amplified from genomic DNA of isolates BL^T and G1^T as well as *T. fructosivorans* ATCC 49749 (=I) and *Thiothrix* sp. DSM 12730 (=CT3) using universal *hsp60* degenerate primers H1612 (5'-GAIHIGCIGGYGACGGYACSAAC-3') and H1613 (5'-CGRCGRTRCCGAAGCCSGGIGCCTT-3') under the PCR conditions given by Hill *et al.* (2006). The expected 550 bp amplification product was purified and sequenced.

Sequences were compared with sequences in the GenBank database via BLAST (Altschul *et al.*, 1997). The analysis revealed the highest sequence similarity with the *hsp60* genes of *T. fructosivorans* Q^T (91.2 and 91.5 %, respectively, for strains BL^T and G1^T) and *Thiothrix* sp. DSM 12730 (89.8 and 85.5 %). Strains BL^T and G1^T shared 88.4 % sequence similarity (Supplementary Table S1).

Table 2. Characteristics that differentiate strains BL^T and G1^T from other *Thiothrix* species

Strains: 1, BL^T 2, G1^T; 3, *T. fructosivorans* Q^T; 4, *T. unzii* A1^T; 5, *T. nivea* JP2^T; 6, *T. flexilis* EJ2M-B^T; 7, *T. disciformis* B3-1^T; 8, *T. eikelboomii* AP3^T. Data were taken from this study and from Williams & Unz (1985), Howarth *et al.* (1999) (*T. unzii* and *T. fructosivorans*), Larkin & Shinabarger (1983) (*T. nivea*) and Aruga *et al.* (2002) (type 021N group). +, Positive; −, negative; ±, weakly positive; ND, no data available.

Characteristic	<i>T. nivea</i> group					Type 021N		
	1	2	3	4	5	6	7	8
Sheath	+	+	+	−	+	−	−	−
Nitrate reduction	+	+	+	+	+	+	−	+
DNA G + C content (mol%)	51.4	52	51.5*	ND	52	44.0–44.4†	43.9–44.7†	44.1–46.1†
Catalase activity	−	−	±	−	−	+	+	+
Assimilation of nitrogen	−	+	−	ND	+	ND	ND	ND
Utilization of carbon sources								
Malate	−	−	+	+	+	ND	ND	+
Fumarate	+	+	+	+	−	ND	ND	+
2-Oxoglutarate	+	−	−	−	ND	ND	ND	+
Formate	−	−	+	+	−	−	−	−
Lactate	+	+	+	+	−	+	−	+
Sucrose, D-fructose	−	−	+	−	−	+	+	+
Maltose, D-glucose, trehalose, mannitol	−	−	−	−	−	+	+	+
Aspartate	+	+	−	−	−	+	+	ND
Glutamate	+	−	−	−	−	+	+	+
Cysteine, cystine, asparagine	+	−	−	−	ND	ND	ND	−
Leucine, isoleucine	−	+	−	−	ND	ND	ND	−

*Data for *T. fructosivorans* I.

†Ranges of values for a number of strains including the type strains (Aruga *et al.*, 2002).

The *gyrB* gene was amplified by PCR using universal *gyrB* primers Up1 (5'-GAAGTCATCATGACCGTTCTGCAY-GCNGGNGGNAARTTYGA-3') and Up2r (5'-AGCAG-GGTACGGATGTGCGAGCCRTCNCACRTCNCGTCAT-3') (Yamamoto & Harayama, 1998). Strains BL^T and G1^T shared 83.1 % sequence similarity and showed

83.6 and 85.4 % *gyrB* sequence similarity, respectively, to *T. fructosivorans* ATCC 49749 and 84.5 % to *Thiothrix* sp. DSM 12730 (Supplementary Table S1).

A fragment of the *nifH* gene was obtained for strains G1^T, G2, P and K2 but not for strain BL^T by PCR using primers F1 (5'-TAYGGIAARGGIAARGGIGGIATIGGIAARTC-3') and *nifH*-3r (5'-TTGTTGGCIGCRTASAKIGCCATT-3'), as described by Fedorov *et al.* (2008).

Fatty acid analysis was done by using the Microbial Identification System (Sherlock; MIDI Inc.) according to standard protocols (Stead *et al.*, 1992). Fatty acid profiles of strains G1^T and BL^T are given in Table 3. The dominant components were C_{16:1}ω7, C_{16:0} and C_{18:1}ω7. The 3-hydroxy fatty acids C_{10:0} 3-OH and iso-C_{15:0} 3-OH were found in strain G1^T only.

DNA base composition was determined by the thermal denaturation method as described previously (Owen & Lapage, 1976). The molar DNA G + C contents of strains BL^T and G1^T were 51.4 and 52 %, respectively, in accordance with values usually reported for members of the genus *Thiothrix* (43–53 mol%) (Aruga *et al.*, 2002).

DNA–DNA relatedness was determined by measuring the renaturation rates of the denatured DNAs at the optimal renaturation temperatures as recommend by De Ley *et al.* (1970). A Pye Unicam SP 1800 spectrophotometer equipped with a thermoprogrammer and hermetically

Fig. 2. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the position of strains G1^T and BL^T among their phylogenetic neighbours. Numbers at branch nodes are bootstrap values expressed as percentages of 1000 replicates (only values above 50 % are shown). Bar, 0.02 substitutions per nucleotide position. The tree was rooted using the sequence of *Achromatium oxaliferum* clone 5 (GenBank accession no. L42543) as an outgroup (not shown).

Table 3. Comparison of the cellular fatty acid contents of strains BL^T, G1^T, *Thiothrix* sp. CT3 and *T. fructosivorans* I

Strains: 1, G1^T; 2, BL^T; 3, CT3 (data obtained in this study for strain DSM 12730); 4, *T. fructosivorans* I (data obtained in this study for strain ATCC 49749). Values are percentages of the total peak area. –, Not detected.

Fatty acid	1	2	3	4
C _{12:0}	–	–	–	1.1
C _{10:0} 3-OH	0.4	–	–	–
iso-C _{14:0}	0.8	–	–	–
C _{14:0}	0.9	–	0.8	2.2
iso-C _{15:0}	1.0	–	–	1.6
anteiso-C _{15:0}	1.2	–	–	1.6
C _{15:0}	–	–	–	1.2
iso-C _{16:0}	1.0	–	–	–
C _{16:1} ω7	38.7	64.5	45.7	39.3
C _{16:0}	26.7	10.9	18.0	22.5
iso-C _{15:0} 3-OH	1.3	–	–	–
C _{18:1} ω7	27.3	24.6	34.2	28.1
C _{18:0}	0.7	–	1.3	2.4

sealed thermocuvettes was used. Strains BL^T and G1^T shared 35 % DNA–DNA relatedness, and respectively showed 51 and 53 % relatedness to *T. fructosivorans* ATCC 49749 (Supplementary Table S2).

The phenotypic and genotypic characteristics described above suggest that strain G1^T and BL^T differ at the species level from previously described species of the genus *Thiothrix* and from each other. Therefore, we propose the names *Thiothrix caldifontis* sp. nov. for strains G1^T, G2, K2 and P and *Thiothrix lacustris* sp. nov. for strain BL^T.

Description of *Thiothrix caldifontis* sp. nov.

Thiothrix caldifontis (cal.di.fon'tis. L. adj. *caldus* hot; L. n. *fons*, *fontis* a spring; N.L. gen. n. *caldifontis* from a hot spring, pertaining to the source of isolation of the first strains).

Rod-shaped cells with rounded ends, seriate in multicellular filaments (trichomes) with polysaccharide sheaths. Gram-negative and aerobic. Cells of the major form are 0.9–2.2 µm in diameter and 3.2–6.5 µm long. Filaments are non-motile. Gliding gonidia are produced from the apical ends of the filaments. Gonidia can form rosettes. At early stages of exponential growth, a spiral form of filaments is often observed. The top cells of short filaments sometimes form pin-like bulges during the stationary growth phase. Colonies are white with fibrous edges, 1–5 mm in diameter. The temperature range for growth is 7–37 °C, with optimum growth at 25 °C. The pH range for growth is 7.0–8.6, with optimum growth at pH 8.0. A number of organic acids, including acetate, lactate, pyruvate, oxaloacetate, fumarate and succinate, and amino acids, including aspartate, leucine and isoleucine, are utilized as carbon and energy sources for chemo-organoheterotrophic growth.

Alcohols, sugars, peptone and casein hydrolysate are not used. Peptone, yeast extract, ammonium, nitrate and nitrite are used as sole nitrogen sources. Anaerobic growth with nitrate does not occur. Capable of nitrogen fixation. Negative in tests for catalase activity and hydrolysis of starch and casein. Positive in tests for oxidase and urease activities. During growth with cysteine or thiosulfate, no sulfide is formed. Capable of lithotrophic growth in the presence of reduced sulfur compounds. Thiosulfate and sulfide are oxidized to sulfate and element sulfur. Maximum cell counts are obtained during mixotrophic growth in the presence of thiosulfate, lactate and other organic substrates. The major fatty acids are C_{16:1}ω7, C_{16:0}, C_{18:1}ω7; among the minor components, the 3-hydroxy fatty acids C_{10:0} 3-OH and iso-C_{15:0} 3-OH are found. The G + C content of the DNA of the type strain is 52 mol% (*T_m*).

The type strain is strain G1^T (=DSM 21228^T =VKM B-2520^T), isolated from the sulfide spring Petushok, at 33–40 °C, in the Northern Caucasus region, Russia. Additional strains are G2, P and K2.

Description of *Thiothrix lacustris* sp. nov.

Thiothrix lacustris (la.cus'tris. N.L. fem. adj. *lacustris* belonging to a lake, referring to the site from where the type strain was isolated).

Rod-like cells with rounded ends occur in sheathed filaments. Gram-negative and aerobic. Cells of the major form are 0.9–2.3 µm in diameter and 4.4–6.3 µm long. Filaments are non-motile. Gliding gonidia are produced from the apical ends of the filaments. Gonidia can produce rosettes. At early stages of exponential growth, a spiral form of filaments is often observed. During the stationary growth phase, pin-like bulges can appear on the ends of short filaments. Colonies are white with fibrous edges, 1–5 mm in diameter. The temperature range for growth is 5–32 °C, with optimum growth at 24 °C. The pH range for growth is 6.2–8.2, with optimum growth at pH 7.0. A number of organic acids, including acetate, lactate, pyruvate, succinate, fumarate, oxalate, oxaloacetate, 2-oxoglutarate, citrate, isocitrate, malonate and aconitate, and amino acids, including aspartate, glutamate, cysteine, cystine and asparagine, are utilized as carbon and energy sources for chemo-organoheterotrophic growth. Alcohols, sugars, peptone and casein hydrolysate are not used. Peptone, yeast extract, ammonium, nitrate and nitrite are used as sole nitrogen sources. Anaerobic growth with nitrate does not occur. Incapable of nitrogen fixation. Negative in tests for catalase activity and hydrolysis of starch and casein. Positive in tests for oxidase and urease activities. During growth with cysteine or thiosulfate, sulfide is not formed. Capable of lithotrophic growth in the presence of reduced sulfur compounds. Thiosulfate and sulfide are oxidized to sulfate and element sulfur. Maximum cell counts are obtained during mixotrophic growth in the presence of thiosulfate, lactate (or other

organic substrates). The major fatty acids are C_{16:1ω7}, C_{16:0} and C_{18:1ω7}. The G+C content of the DNA of the type strain is 51.4 mol% (*T_m*).

The type strain is strain BL^T (=DSM 21227^T =VKM B-2521^T), isolated from a low-temperature lake of the Blue lake system (Kabardino-Balkaria).

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