Sulfurivermis fontis gen. nov., sp. nov., a sulfur-oxidizing autotroph, and proposal of Thioprofundaceae fam. nov.

Hisaya Kojima,1,* Miho Watanabe1,2 and Manabu Fukui1

Abstract

A novel Gram-stain-negative, chemolithoautotrophic sulfur oxidizer, strain JG42T, was isolated from a hot spring microbial mat. As an electron donor for autotrophic growth, strain JG42T utilized sulfide, thiosulfate, tetrathionate and elemental sulfur. Cells of strain JG42T were oxidase-positive and catalase-negative. The G+C content of the genomic DNA was 65 mol%. The predominant cellular fatty acid was C16:0. Phylogenetic analysis of the 16S rRNA gene indicated that strain JG42T belonged to the order Chromatiales, but sequence similarities to the known species were less than 94%. On the basis of its properties, strain JG42T (=DSM 104776T=NBRC 112696T) is proposed as the type strain of a novel species of a new genus, Sulfurivermis fontis gen. nov., sp. nov., which belongs to the family Thiokalkalispiraceae. A new family, Thioprofundaceae fam. nov., is also proposed to accommodate the genus Thioprofundum, transferred from the family Thiokalkalispiraceae.

The order Chromatiales includes many phototrophic and chemolithotrophic sulfur oxidizers. Among the seven families in this order, the families Halothiobacillaceae and Thiokalkalispiraceae consist of chemolithotrophic sulfur oxidizers [1, 2], whereas the family Ectothiorhodospiraceae encompasses both phototrophic and chemotrophic sulfur-oxidizing bacteria [3]. In addition, a sulfur-oxidizing chemolithoautotroph was recently described as a new member of the family Granulosicoccaceae [4], which had until then consisted of only obligately chemoheterotrophic species [5]. In this study, a novel sulfur-oxidizing chemolithoautotroph was isolated and characterized to be proposed as the type species of a new genus in the order Chromatiales.

The novel isolate, strain JG42T, was isolated from an enrichment culture from which Sulfuritortus calidifontis J1A1T was isolated [6]. The enrichment culture was obtained with a medium containing elemental sulfur and nitrate as an electron donor and acceptor, respectively. A microbial mat from Jozankei hot spring (42°57′53″N 141°09′47″E) in Japan was used as an inoculum of this enrichment culture established at 45°C. The basal medium of the following composition (hereafter referred to as S5 medium) was used throughout this study, unless otherwise specified (per litre): 2.5 g Na2S2O3·5H2O, 0.5 g MgSO4·7H2O, 0.1 g CaCl2·2H2O, 0.1 g NH4Cl, 0.1 g KH2PO4, 0.1 g KCl, 1 ml vitamin mixture solution (DSM 141), 1 ml trace element solution, 1 ml selenium/tungstate solution and 30 ml NaHCO3 solution (1 M). Solutions of the last three components were prepared as described previously [7]. To induce changes in microbial community structure, a portion of the enrichment culture was transferred to the S5 medium supplemented with 10 mM nitrate and cultured at increased temperature. The cultivation was performed at 50°C under anoxic conditions created by filling the headspace of the culturing bottles with a mixed gas (N2/CO2: 80:20, v/v, 100 kPa total pressure). After several transfers under the same conditions, the culture medium was further changed to one with a higher salt concentration. The high-salt medium contained 20 g NaCl, 3 g MgCl2·6H2O and MgSO4·7H2O were changed to 5 and 0.3 g l−1, respectively). Culturing with this medium was performed at 45°C without shaking, in closed bottles with the headspace filled with air at atmospheric pressure. From the resulting enrichment culture, an isolate designated as strain JG42T was obtained by repeated agar shake dilution under anoxic conditions [7], using the high-salt medium supplemented with 10 mM nitrate. The final concentration of agar was 1.1% (w/v). The purity of the isolate was checked by microscopy and repeated sequencing of the 16S rRNA gene.

For the characterization of strain JG42T, all culturing experiments were performed at 45°C under oxic conditions (as described above) using S5 medium, unless otherwise specified.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain is LC225746.

Two supplementary figures are available with the online Supplementary Material.

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The Gram-stain test was performed with a staining kit (Fluka). Catalase activity was assessed with 3% H₂O₂ solution and oxidase activity was tested with a test reagent (bio-Mérieux). The G+C content of the genomic DNA was determined by HPLC [8], using a kit from Yamasa Shoyu. The analysis of cellular fatty acids was carried out at Techno Suruga, with cells grown at 45°C for 3 days. The fatty acid profile was analysed by using the Sherlock Microbial Identification System version 6.0 (MIDI), with TSBA6 database.

Utilization of electron donors was tested under aerobic conditions, with modified S5 medium without thiosulfate. Utilization of carbon sources was tested with S5 medium without bicarbonate, buffered with 20 mM MOPS. Anaerobic growth was tested with S5 medium supplemented with nitrate or nitrite in closed bottles with headspace filled with N₂/CO₂ (80:20, v/v, 100 kPa total pressure). Heterotrophic growth in complex liquid media was tested for the following media and those supplemented with 2% (w/v) NaCl: R2A (Daigo), diluted (1/10) R2A, NB (Difco) and TSB (Oxoid).

The 16S rRNA gene of strain JG42 was amplified by PCR, using primers 27F and 1492R [10] and then directly sequenced by using a BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems). The obtained gene sequence was aligned with reference sequences retrieved from the GenBank/EMBL/DDBJ database, by using the program CLUSTAL X version 2.1 [11]. The reference sequences included 51 representatives from all families of the order Chromatiales and four environmental clone sequences which have 97% or higher sequence similarity with strain JG42 T, identified by a BLAST search. All positions with gaps were excluded from the calculation, and 1091 positions were used for the following analyses. Evolutionary distances were computed using the maximum composite likelihood method. Phylogenetic trees were reconstructed by using the neighbour-joining and minimum-evolution methods with the program MEGA version 7.0.20 [12].

Cells of strain JG42 T were curved rods, 0.4–0.6 µm in width and 1.5–12 µm in length (Fig. S1, available in the online Supplementary Material). Cells were motile, Gram-stain-negative, catalase-negative and oxidase-positive. The G+C content of the genomic DNA was 65 mol% (HPLC).

Growth of strain JG42 T was observed over a temperature range between 25 and 50°C, with optimum growth at 42–48°C. The range of pH for growth was 6.1–8.9, and the optimum pH was 7.2–7.9. Strain JG42 T exhibited optimum growth in the presence of 0–1% (w/v) NaCl, and did not grow in the presence of 3% or more NaCl.

In the cellular fatty acid profile of the strain, C₁₆:₀ was the predominant fatty acid, accounting for 47% of the total. The other fatty acids detected were summed feature 3 (C₁₆:₁ω7c and/or C₁₆:₁ω6c, 23.9%), summed feature 8 (C₁₈:₁ω7c and/or C₁₈:₁ω6c, 13.0%), summed feature 9 (iso-C₁₇:₁ω7c and/or C₁₆:₀ 10-methyl; 6.2%), C₁₄:₀ (4.7%), C₁₀:₀ 3-ОH (1.5%), C₁₂:₀ 3-ОH (1.2%), C₁₂:₀ (0.5%), C₁₈:₁ω9c (0.5%), C₁₇:₀ (0.4%), C₁₆:₁ω5c (0.3%) and C₁₄:₁ 3-ОH (0.1%).

Strain JG42 T was a facultative anaerobe that can use nitrate (10 mM) as a terminal electron acceptor to support growth, but not nitrite (1 and 5 mM). Chemolithoautotrophic growth of strain JG42 T was supported by thiosulfate (10 mM), thionatate (10 mM), sulfide (2 mM) and elemental sulfur (0.5 g 1⁻¹), but sulfite (5 mM) and hydrogen (air/H₂, 50:50, v/v; 200 kPa total pressure) did not support growth. The following substrates did not support heterotrophic growth of strain JG42 T: acetate, lactate, fumarate, succinate, malate, benzoate, butyrate, isobutyrate, formate, D-glucose, D-sorbitol, mannose, D-xyllose, L-arabinose and N-acetylglucosamine (all 5 mM). Strain JG42 T exhibited no growth on R2A, diluted R2A, NB or TSB, and addition of 2% NaCl did not affect the results. In the presence of thiosulfate, aerobic growth was observed in medium containing bicarbonate, but the following compounds did not serve as carbon source to support growth under the same conditions: acetate, lactate, succinate, malate, benzoate, butyrate, isobutyrate, formate, D-glucose, D-sorbitol, mannose, D-xyllose, L-arabinose and N-acetylglucosamine (all 5 mM). Strain JG42 T was observed in medium containing nitrate or ammonium as the sole nitrogen source, and was inhibited by kanamycin and ampicillin.

Analysis of the 16S rRNA gene revealed that strain JG42 T is a relative of species in the order Chromatiales, but sequence similarities with these species were less than 94%. Uncultured bacterial clones with the highest sequence similarity to strain JG42 T were detected in a bioreactor for wastewater treatment [13] and water sample from an oil well [14]. By reconstructing phylogenetic trees, it was confirmed that strain JG42 T is a member of the order Chromatiales (Figs 1 and S2). In the trees of identical topology obtained with the neighbour-joining and minimum-evolution methods (Fig. 1), strain JG42 T and related clones formed a cluster with the genera Thioalkalispira [15] and Thiohalophilus [16]. These genera belong to the family Thioalkalispiraceae, but the other members of this family (Thiopseudomonad species) were positioned apart from strain JG42 T in the phylogenetic trees (Fig. 1). A different tree was obtained with the maximum-likelihood method (Fig. S2), but strain JG42 T...
All phylogenetic trees reconstructed suggested that strain JG42<sup>T</sup> in this tree.

All phylogenetic trees reconstructed suggested that strain JG42<sup>T</sup> should be classified into the family Thialkalispiraceae, although it was not fully supported by the bootstrap analyses (between 40 and 50 % in all trees). At this point, strain JG42<sup>T</sup> can only be regarded as a member of the family Thialkalispiraceae. On the other hand, the phylogenetic isolation of the genus Thioprofundum in this family was also indicated (Fig. 1). In fact, the polyphyly of the family Thialkalispiraceae has been repeatedly observed in previous phylogenetic trees [4, 17, 18]. To solve this problem, a new family, Thioprofundaceae fam. nov., is proposed to accommodate the genus Thioprofundum. These taxonomic assignments are not supported by solid evidence at present, but seem to be the best options to avoid further confusion in classification within the order Chromatiales. The neighbour-joining and minimum-evolution phylogenetic trees also indicated that the existing genera in the family Thialkalispiraceae cannot accommodate strain JG42<sup>T</sup> without disrupting the monophyly (Fig. 1). In addition to the low sequence similarities (<94 %), phenotypic properties of the novel strain were also distinct from those of strains representing the type genus of the family; suff. -aceae ending to denote a family; N.L. fem. pl. n. Thioprofundaceae the family of the genus Thioprofundum).

**DESCRIPTION OF THIOPROFUNDACEAE FAM. NOV.**

Thioprofundaceae (Thi.o.pro.fun.da.ce.a. N.L. neut. n. Thioprofundum the type genus of the family; suff. -aceae ending to denote a family; N.L. fem. pl. n. Thioprofundaceae the family of the genus Thioprofundum).
The cell wall is of Gram-negative type. The phylogenetic position is in the order Chromatiales within the class Gammaproteobacteria of the phylum Proteobacteria. The type genus of the family is Thioprofundum.

DESCRIPTION OF SULFURIVERMIS GEN. NOV.
Sulfurivermis (Sul.fu.ri. ver’mis. L. neut. n. sulfur sulfur; L. masc. n. vermis worm. N.L. masc. n. Sulfurivermis sulfur-oxidizing worm).

Grow chemolithoautotrophically by the oxidation of inorganic sulfur compounds. Gram-stain-negative. Major cellular fatty acid is C_{16:0}. Based on 16S rRNA gene sequence analysis, affiliated to the family Thioalkalispiraceae in the order Chromatiales. The type species is Sulfurivermis fontis.

DESCRIPTION OF SULFURIVERMIS FONTIS SP. NOV.
Sulfurivermis fontis (fon’tis. L. masc. gen. n. fontis of a spring).

Cells are motile, 0.4–0.6 μm wide and 1.5–12 μm long. Facultatively anaerobic and reduces nitrate as an electron acceptor to support growth. Chemolithoautotrophic growth occurs with oxidation of sulfide, thiosulfate, tetrathionate and elemental sulfur. Nitrate and ammonium are utilized as a nitrogen source. Oxidase-positive and catalase-negative. The temperature range for growth is 25–50 °C, with an optimum of 42–48 °C. The pH range for growth is 6.1–8.9, with an optimum of pH 7.2–7.9. No growth occurs in the presence of 3 % (w/v) NaCl.

The type strain, JG42^T (=DSM 104776^T=NBRC 112696^T), was isolated from a microbial mat of a hot spring in Japan. The G+C content of the genomic DNA of the type strain is 65 mol% (HPLC).

Table 1. Differential properties between strain JG42^T and strains representing related genera

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tr>
<td>DNA G+C content (mol%)</td>
<td>65</td>
<td>58.9</td>
<td>58.2</td>
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<td>Growth at:</td>
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<tr>
<td>pH 7</td>
<td>+</td>
<td>−</td>
<td>+</td>
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<tr>
<td>pH 10</td>
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<tr>
<td>1 M NaCl</td>
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<td>+</td>
<td>+</td>
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<tr>
<td>Growth based on nitrate reduction</td>
<td>+</td>
<td>−</td>
<td>−</td>
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</table>

The authors declare that there are no conflicts of interest.

References

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Conflicts of interest
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