A chemolithotrophic sulfur-oxidizing bacterium, strain aks$^T$, was isolated from sediment of a brackish lake in Japan. The cells were curved rod-shaped and Gram-stain-negative. The G+C content of the genomic DNA was 53 mol%. The major components in the cellular fatty acid profile were C_{16:0} and summed feature 3 (C_{16:1ω7c} and/or C_{16:1ω6c}). As electron donor for chemolithoautotrophic growth, strain aks$^T$ oxidized thiosulfate, sulfide, and elemental sulfur. The strain could utilize oxygen and nitrate as an electron acceptor for thiosulfate oxidation. Growth was observed at a temperature range of 5–34 °C, with optimal growth at 30–32 °C. Growth of the strain was observed at a pH range of 6.4–8.7. Phylogenetic analysis based on 16S rRNA gene sequences indicated that the strain is related to members of the family Granulosicoccaceae within the order Chromatiales, with sequence similarities around 92%. On the basis of phylogenetic and phenotypic properties, strain aks$^T$ represents a novel species of a new genus, for which the name *Sulfuriflexus mobilis* gen. nov., sp. nov. is proposed. The type strain of the type species is aks$^T$ (=DSM 102939 =NBRC 111889$^T$).

**Sulfuriflexus mobilis** gen. nov., sp. nov., a sulfur-oxidizing bacterium isolated from a brackish lake sediment

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The original description of the order Chromatiales contains three families, Chromatiaceae, Ectothiorhodospiraceae and Halothiobacillaceae (Imhoff, 2005), and four families, Granulosicoccaceae (Lee et al., 2007), Thioalkalispiraceae (Mori et al., 2011), Wenzhouxiangellaceae (Wang et al., 2015), and Woesiaceae (Du et al., 2016) have subsequently been added in the order. In the present study, a novel chemolithoautotrophic sulfur-oxidizing bacterium, strain aks$^T$, was isolated and characterized as a representative of the type species of a new genus in this order.

Strain aks$^T$ was isolated from sediment of a brackish lake (Lake Akkeshi) in Japan. Throughout this study, a bicarbonate-buffered defined medium was used as basal medium. To prepare the medium, the following constituents (l$^{-1}$) were dissolved in distilled water and then autoclaved: 20 g NaCl, 3 g MgCl$_2$ · 6H$_2$O, 0.3 g MgSO$_4$ · 7H$_2$O, 0.1 g CaCl$_2$ · 2H$_2$O, 0.1 g NH$_4$Cl, 0.1 g KH$_2$PO$_4$ and 0.1 g KCl. After cooling to room temperature, 1 ml trace element solution, 1 ml selenite-tungstate solution, 30 ml NaHCO$_3$ solution and 1 ml vitamin mixture solution (DSM 141) were aseptically added to the main body of medium. The solutions of trace element, selenite-tungstate and NaHCO$_3$ were prepared as described previously (Widdel & Bak, 1992). Before dispensing into culture containers, the pH of the medium was adjusted to 7.0–7.2. The enrichment culture was established with the basal medium supplemented with elemental sulfur (ca. 0.5 g l$^{-1}$). After 11 transfers to fresh medium of the same composition (1–2%), the sole electron donor was changed to 20 mM Na$_2$S$_2$O$_3$. Finally, the strain was isolated in pure culture by repeated serial dilution in the medium supplemented with Na$_2$S$_2$O$_3$. Purity of the isolate was checked by microscopy and sequencing of the 16S rRNA gene fragments amplified by using some PCR primer pairs as described previously (Higashioka et al., 2012).

For the characterization of the strain, the basal medium supplemented with 20 mM Na$_2$S$_2$O$_3$ was used unless otherwise specified. Culturing experiments were performed in bottles closed with rubber stoppers, and the bottles were incubated without shaking at 30 °C unless otherwise specified.

The Gram-stain test was conducted with a kit (Fluka), and oxidase activity was investigated by using an oxidase test reagent (bioMérieux). Catalase activity was assessed by pouring 3% H$_2$O$_2$ solution onto a pellet of cells. The genomic G+C content of the DNA was determined with HPLC methods (Katayama-Fujimura et al., 1984), using a kit (Yamasaki Shoyu). The fatty acid profile of the strain was

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One supplementary figure is available with the online Supplementary Material.

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The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain is LC131141.
Cells of strain aks1<sup>T</sup> were motile curved rods, 0.9–6.0 µm long and 0.3–0.5 µm wide (Fig. 1). Strain aks1<sup>T</sup> was Gram-stain-negative, catalase-negative and oxidase-positive. The G+C content of the genomic DNA assessed by the HPLC-based method was 53 mol%. In the cellular fatty acid profile, major components were summed feature 3 (C<sub>16:1</sub>ω7c and/or C<sub>16:1</sub>ω6c; 57.3 %) and C<sub>16:0</sub> (32.2 %). The other fatty acids detected were summed feature 9 (iso-C<sub>17:1</sub>ω7c and/or C<sub>16:0</sub>10-methyl; 5.2 %), summed feature 8 (C<sub>18:1</sub>ω7c and/or C<sub>18:1ω6c</sub>; 1.7 %), C<sub>10:0</sub> 3-OH (1.3 %), C<sub>18:0</sub> (0.8 %), C<sub>14:0</sub> (0.6 %), C<sub>12:0</sub> 3-OH (0.4 %), C<sub>17:0</sub> (0.3 %) and C<sub>10:0</sub> (0.2 %).

Growth of strain aks1<sup>T</sup> was observed over a temperature range between 5 °C and 34 °C, with optimal growth at 30–32 °C; at a pH range of 6.4–8.7, with optimal growth at pH 6.8–8.3; and in medium with 1–4 % NaCl with an optimum of 3 %.

The isolate grew chemolithothrophically on thiosulfate (20 mM), sulfide (2 mM) and elemental sulfur (0.5 g l<sup>−1</sup>). Hydrogen gas (air/H<sub>2</sub>; 4:1, v/v; 125 kPa total pressure), tetrationate (20 mM) and sulfite (5 mM) did not support autotrophic growth of the strain. The following organic substrates did not support growth of strain aks1<sup>T</sup>: pyruvate (5 mM), lactate (5 mM), acetate (5 mM), methanol (5 mM), succinate (2.5 mM), fumarate (2.5 mM), butyrate (2.5 mM), isobutyrate (2.5 mM), ethanol (2.5 mM), formate (5 mM), lactose (2.5 mM), glucose (2.5 mM) and xylose (2.5 mM). The strain exhibited no growth on MB or other complex media tested. Nitrate (20 mM) supported anaerobic growth of the strain as sole electron acceptor for thiosulfate oxidation.

Among characterized strains of species with validly published names, Thioprofundum hispidum gsp61<sup>T</sup> showed the highest 16S rRNA gene sequence similarity to strain aks1<sup>T</sup> (93 %), followed by the type strain of the species Granulosicoccus (92 %) and some other bacteria in the order Chromatiales. By reconstructing phylogenetic trees, it was revealed that strain aks1<sup>T</sup> forms a monophyletic cluster with species of the genus Granulosicoccus (Figs. 2, S1, available in the online Supplementary Material). The methods of neighbour-joining and minimum-evolution generated trees of identical topology (Fig. 2), but a different tree was obtained with the maximum-likelihood method (Fig. S1). In all trees, strain aks1<sup>T</sup> represents a sister group of the genus Granulosicoccus, and they form a cluster with the genera Thioalkalispira and Thiohalophilus. The genus Granulosicoccus is the sole genus in the family Granulosicoccaceae, whereas Thioalkalispira and Thiohalophilus belong to the family Thioalkalispiraceae. The other genus of the family Thioalkalispiraceae, the genus Thioprofundum, was positioned apart from these genera (Figs 2, S1). This phylogenetic isolation of Thioprofundum from the other genera was also shown in previously reconstructed phylogenetic trees (Mori & Suzuki, 2014; Mori et al., 2015), suggesting that the family Thioalkalispiraceae is not monophyletic. As pointed out previously, it is difficult to clarify phylogenetic relationships among the families in the order Chromatiales (Mori et al., 2015), and reclassification of some taxa may be required in the future. However, it seems reasonable to place strain aks1<sup>T</sup> in the family Granulosicoccaceae at this point.
Differential properties of strain aks1T and related genera are summarized in Table 1. In contrast to heterotrophic species of the genus Granulosicoccus, growth of strain aks1T was not observed in complex media including MB, or synthetic medium supplemented with organic substrates. Differences between strain aks1T and species of the genus Granulosicoccus are apparent in cell morphology, oxygen requirement and catalase activity (Table 1). On the basis of the distinct phenotypic properties and isolated phylogenetic position, strain aks1T is proposed to represent a novel species of a new genus in the family Granulosicoccaceae, with the name Sulfuriflexus mobilis gen. nov., sp. nov.

**Description of Sulfuriflexus mobilis gen. nov.**

*Sulfuriflexus* (Sul.fu.ri.flex'u.xus. L. neut. n. sulfur sulfur; L. masc. n. flexus a bending. N.L. masc. n. Sulfuriflexus sulfur-oxidizing bending).

Cells are motile and Gram-stain-negative. Grows chemolithoautotrophically by the oxidation of inorganic sulfur compounds. Belongs to the family Granulosicoccaceae as determined by 16S rRNA gene sequence analysis.

The type species is *Sulfuriflexus mobilis*.

**Description of Sulfuriflexus mobilis sp. nov.**

*Sulfuriflexus mobilis* (mo'bi.lis. L. masc. adj. mobilis movable, motile).

Cells are curved rod-shaped, 0.9–6.0 µm in length and 0.3–0.5 µm in width. Autotrophic growth occurs with oxidation of thiosulfate, sulfide and elemental sulfur. Oxidase-positive and catalase-negative. Growth occurs at temperatures of 5–34°C with optimum growth at 30–32°C. The pH range for growth is 6.4–8.7. Major cellular fatty acids are C₁₆:0 and summed feature 3 (C₁₆:1ω₇c and/or C₁₆:1ω₆c).

The type strain aks1T (=DSM 102939T=NBRC 111889T) was isolated from sediment of a brackish lake in Japan (Lake Akkeshi). The G+C content of genomic DNA of the type strain is 53 mol%.

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**Fig. 2.** Minimum-evolution tree showing the phylogenetic position of strain aks1T within the order Chromatiales based on 16S rRNA gene sequence analysis. This tree was reconstructed using ca. 1200 sites. *Sulfuricaulis limicola* and *Acidiferrobacter thiooxydans* are included as outgroup species. The neighbour-joining method yielded a tree of identical topology. Numbers at nodes represent percentage values of 1000 bootstrap resamplings; values <50 are not shown. Bar, 0.01 substitutions per nucleotide position.
Table 1. Differential properties of strain aks1T and related genera

Genera: 1, Granulosicoccus (data compiled from descriptions of type strains of four species of this genus, taken from Baek et al., 2014; Park et al., 2014; Kurilenko et al., 2010; Lee et al., 2007); 2, Thioalkalispira (Sorokin et al., 2002); 3, Thiohalophilus (Sorokin et al., 2007). ND, No data available.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>aks1T</th>
<th>Genera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell morphology</td>
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<td>Coccoid</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
<td>+/–*</td>
</tr>
<tr>
<td>Heterotrophic growth</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Anaerobic growth</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Catalase</td>
<td>–</td>
<td>+</td>
</tr>
</tbody>
</table>

*One of the four species is non-motile and the others are motile.

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References


