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Title	Sulfuricaulis limicola gen. nov., sp nov., a sulfur oxidizer isolated from a lake
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Supplementary material

***Sulfuricaulis limicola* gen. nov., sp. nov., a novel sulfur oxidizer isolated from a lake**

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Procedures for enrichment and isolation

The first enrichment culture was established with the medium S1, inoculated with the sediment sample. As a sole electron donor, elemental sulfur (ca. 0.5 g l⁻¹) was added in the medium. After 3 times transfer to the fresh medium (1–2%), the resulting fourth culture was inoculated into the medium S2 which was also supplemented with elemental sulfur. After 3 times transferring with the medium S2, culturing medium was further changed to the medium S3 supplemented with elemental sulfur. From the established sulfur-oxidizing enrichment culture in the medium S3, the isolate was obtained by agar shake dilution using medium S2 supplemented with 20 mM thiosulfate. The head space of the agar shake tubes were filled with mixture gas of N₂/CO₂ (80 : 20, v/v), but no reductant was added to the medium and thus dissolved oxygen was not eliminated. A colony was picked up and maintained in the medium S4 supplemented with 20 mM sodium thiosulfate. All cultivation for enrichment and isolation was carried out in the dark at 28°C.

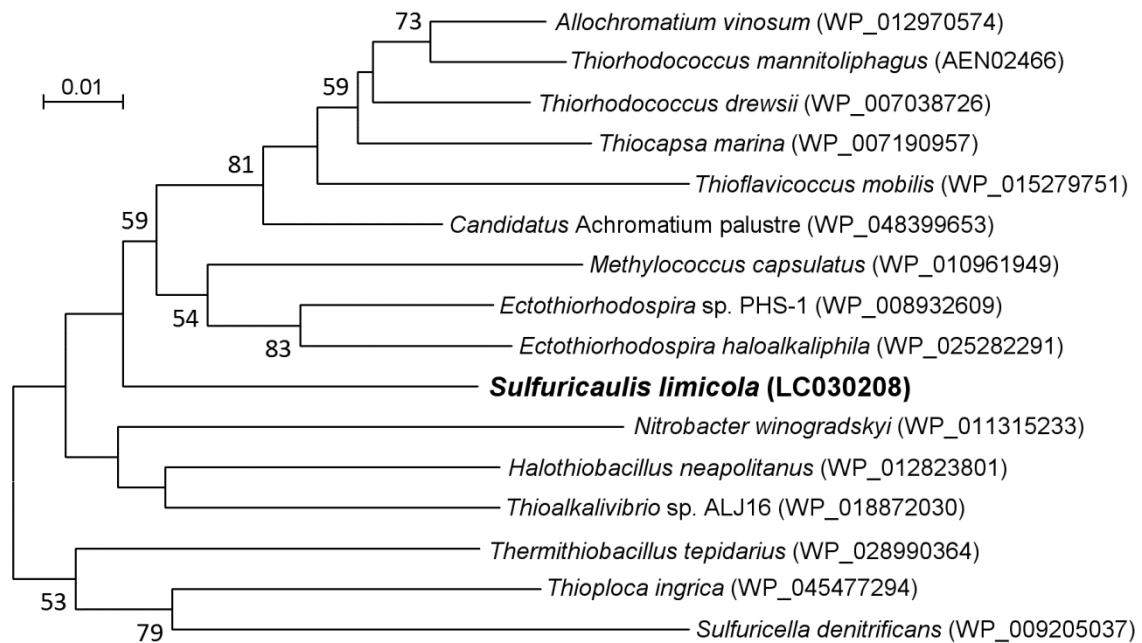


Fig. S1. Phylogenetic position of the CbbL amino acid sequence of *Sulfuricaulis limicola*. This tree was constructed with the neighbor-joining method (321 amino acid positions were used) with 1000 bootstrap resamplings (bootstrap values > 50% are shown at the nodes). Bar, 0.01 substitutions per amino acid position.



Fig S2. DGGE profile of the *aprA* genes of *Sulfuricaulis limicola* HA5^T.

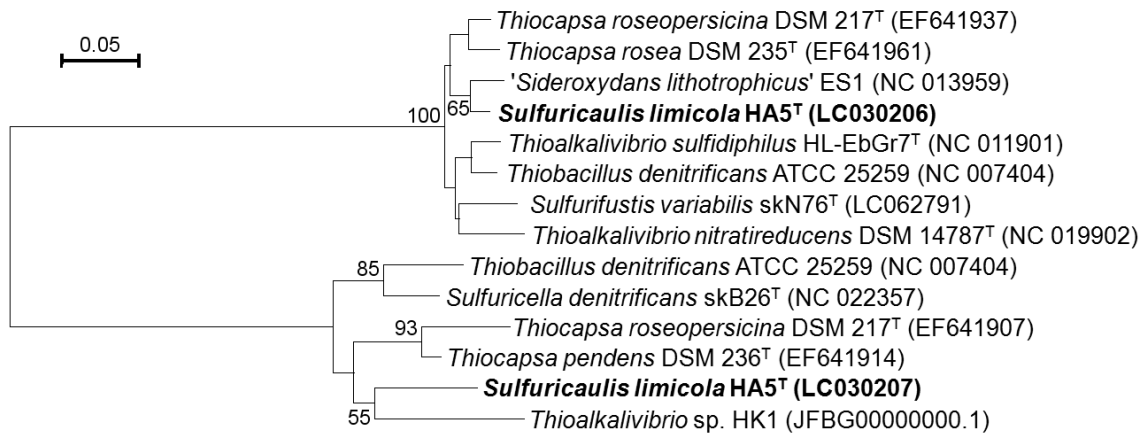


Fig. S3. Phylogenetic positions of the AprA amino acid sequences of *Sulfuricaulis limicola* HA5^T, deduced from nucleotide sequences of the DGGE bands. This tree was constructed with the neighbor-joining method (119 amino acid positions were used) with 1000 bootstrap resamplings (bootstrap values > 50% are shown at the nodes). Bar, 0.05 substitutions per amino acid position.