Reclassification of *Sulfolobus hakonensis* Takayanagi *et al.* 1996 as *Metallosphaera hakonensis* comb. nov. based on phylogenetic evidence and DNA G + C content

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The taxonomic status of *Sulfolobus hakonensis* Takayanagi *et al.* 1996 was re-evaluated by fresh determinations of the 16S rDNA sequence and G + C content of the genomic DNA of the type strain, HO1-1^T. The 16S rDNA sequence of strain HO1-1^T showed 98 % similarity to those of two *Metallosphaera* species and only ≤92 % similarity to those of other *Sulfolobus* species. The DNA G + C content (46.2 mol%) is in accordance with those of *Metallosphaera* species. In addition, strain HO1-1^T shares some phenotypic properties with *Metallosphaera* species; however, it can be differentiated from them by its capacity to utilize FeS and tetrathionate and the absence of flagella. Therefore, it is proposed that *Sulfolobus hakonensis* should be transferred to the genus *Metallosphaera* as *Metallosphaera hakonensis* comb. nov.

Sulfolobus hakonensis was proposed by Takayanagi *et al.* (1996) for a thermoacidophilic organism isolated from a hot spring in Ohwaku-dani, Hakone, Kanagawa, Japan. Strain HO1-1^T is the sole reported member of the species and was described as a lobe-shaped coccus, thriving under strictly aerobic and thermoacidophilic conditions (pH 3.0 and 70 °C) and with a G + C content of genomic DNA of 38.4 mol%. In addition to these properties, the presence of calditoglycerocaldoarchaeol and the phylogenetic position deduced from the 16S rDNA sequence suggested its placement in the order *Sulfolobales* (Takayanagi *et al.*, 1996). When the species was proposed, the order *Sulfolobales* (family *Sulfolobaceae*) was composed of only four genera, *Acidianus*, *Desulfurolobus*, *Metallosphaera* and *Sulfolobus*. Among these genera, the monospecific genus *Desulfurolobus* was reclassified to the genus *Acidianus* (Fuchs *et al.*, 1996). The remaining three genera could be distinguished by, for example, their capacity for anaerobic growth and the G + C content of genomic DNA as follows: *Acidianus*, facultative anaerobes with 30–33 mol% G + C; *Metallosphaera*, strict aerobes with 45–47 mol% G + C; *Sulfolobus*, strict aerobes with 34–39 mol% G + C. Takayanagi *et al.* (1996) therefore regarded strain HO1-1^T as a member of the genus *Sulfolobus* and compared it with other *Sulfolobus* species

(*Sulfolobus acidocaldarius*, *Sulfolobus solfataricus*, *Sulfolobus shibatae* and *Sulfolobus metallicus*) to justify their proposal of the species *S. hakonensis*.

More recently, as a number of 16S rRNA/DNA sequences of archaeal strains, including those of the order *Sulfolobales*, have become available, phylogenetic analysis has revealed that the currently described species of the genus *Sulfolobus* can be separated into at least three branches that may correspond to different genera (Fuchs *et al.*, 1996; Huber & Stetter, 2001). One branch includes the type species, *S. acidocaldarius*, another includes *S. solfataricus* and *S. shibatae*, while the third is represented by *S. metallicus*. In addition, *Sulfolobus hakonensis* is placed in the vicinity of *Metallosphaera sedula* (Huber *et al.*, 1989) and *Metallosphaera prunae* (Fuchs *et al.*, 1995).

In an attempt to re-evaluate the taxonomic status of the species *S. hakonensis*, we determined almost the entire 16S rDNA sequence (1461 bp) and the G + C content of the genomic DNA of the type strain, HO1-1^T (a generous gift from S. Takayanagi). The DNA base composition was estimated by the HPLC method of Tamaoka & Komagata (1984). The 16S rDNA sequence we determined (accession no. D86414) is essentially identical to that reported previously (accession no. D14052), but the ambiguous bases (40 positions) of the previously reported sequence were resolved. The new 16S rDNA sequence of HO1-1^T showed 98 % similarity to those of the two *Metallosphaera* species, but showed very low similarity to the other members of

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The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of strain HO1-1^T is D86414.

Fig. 1. Phylogenetic tree based on 16S rDNA sequences. The tree was constructed by the maximum-likelihood method (Felsenstein, 1981) using the PHYLIP program (Felsenstein, 1993). Strain and sequence accession numbers (in parentheses) are indicated. The crenarchaeon *Aeropyrum pernix* K1^T (accession no. D83259) was used as the outgroup. All sites with gaps in any sequences were removed from the alignment that was used for estimating pairwise distances. A total of 1391 positions were used for construction of the phylogenetic tree.

the order *Sulfolobales* ($\leq 92\%$). On a phylogenetic tree constructed by the maximum-likelihood method (Felsenstein, 1981), strain HO1-1^T is clustered with the two *Metallosphaera* species (Fig. 1). The tree topology, particularly the position of strain HO1-1^T, was identical to that of a previous tree (Huber & Stetter, 2001) and was also supported by the neighbour-joining method (Saitou & Nei, 1987) (not shown). On the other hand, the G+C content of the genomic DNA determined by us was 46.2 mol% (originally reported as 38.4 mol%). It is thought that the difference (about 8 mol%) between our result and the earlier one originates from the different methods used. The G+C content presented by Takayanagi *et al.* (1996) was estimated by the CsCl isopycnic centrifugation method, which is much less accurate than the HPLC method. The new value for the G+C content of strain HO1-1^T is again closer to those of *Metallosphaera* species than to known *Sulfolobus* species (34.6–42.0 mol%). In agreement with the two *Metallosphaera* species, strain HO1-1^T is a strictly aerobic, facultative chemolithotroph that grows optimally at around 70–75 °C and pH 3. Strain HO1-1^T and the two *Metallosphaera* species utilize complex organic substances such as yeast extract, but not sugars, as carbon sources. Thus, on the basis of the phylogenetic and phenotypic properties described above, it is evident that strain HO1-1^T belongs to the genus *Metallosphaera*. Strain HO1-1^T and the two *Metallosphaera* species also utilize elemental sulfur and several reduced sulfur compounds as energy sources; however, strain HO1-1^T additionally utilizes FeS and tetrathionate, unlike the two *Metallosphaera* species.

Strain HO1-1^T does not have flagella, while *M. prunae* does. Therefore, strain HO1-1^T represents a species separate from the two *Metallosphaera* species and we propose a new combination, *Metallosphaera hakonensis* (Takayanagi *et al.* 1996) comb. nov., with the type strain HO1-1^T.

Description of *Metallosphaera hakonensis* comb. nov.

Metallosphaera hakonensis (ha.ko.nen'sis. N.L. fem. adj. *hakonensis* pertaining to Hakone, Japan, where the type strain was isolated).

Basonym: *Sulfolobus hakonensis* Takayanagi *et al.* 1996.

The description is the same as that given for *Sulfolobus hakonensis* (Takayanagi *et al.*, 1996) except for the DNA G+C content of the type strain, which is 46.2 mol%. The new accession number for the 16S rRNA gene of the type strain is D86414. The type strain, HO1-1^T, is deposited in several culture collections as IAM 14250^T, JCM 8857^T, DSM 7519^T and ATCC 51241^T.

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