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Order

Proteobacteria/Gammaproteobacteria/

Thiohalospirales ord. nov.

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Thi.o.ha.lo.spir.al'es N.L. fem. n. *Thiohalospira*, the type genus of the order; L. fem. pl. n. suff. *-ales*, ending to denote an order; N.L. fem. pl. n. *Thiohalospirales*, the order of the genus *Thiohalospira*.

The order *Thiohalospirales* accommodates aerobic, chemolithoautotrophic, sulfur-oxidizing halophilic bacteria from hypersaline habitats which assimilate CO_2 via the Calvin–Benson-Bassham cycle. The order is a deeply branching lineage of the *Gammaproteobacteria* and consists of a single family *Thiohalospiraceae* and genus *Thiohalospira*. The order-level status was established by phylogenomic analysis based on the Genome Taxonomy DataBase classification.

Type genus: **Thiohalospira** Sorokin et al. 2008^{VP}.

The original phylogenetic placement of the genus *Thiohalospira* into the order *Chromatiales* within the *Gammaproteobacteria* was based on 16S rRNA sequence comparisons. A more recent phylogenomic analysis based on comparison of 120 single copy conserved bacterial marker proteins revealed that *Thiohalospira* forms a deeply branching phylogenetic lineage at the level of a separate order in the *Gammaproteobacteria* (Figure 1). The only currently available genome of this group belongs to the type strain *Thiohalospira halophila* HL 3^{T}. In various phylogenomic reconstructions, it consistently represents a deeply branching lineage that has no closely related metagenome-assembled genomes (MAGs).

We analyzed the average amino acid identity (AAI) between *Thiohalospira halophila* HL 3^{T} (GCF_900112605.1) and all available genomes of the cultured members of the *Gammaproteobacteria* with the highest values shown in Table 1. The relative number of proteins involved in the analysis is in the range 39–49% of all genes in the genomes (1,270 genes on average), which we consider to be sufficient for a robust comparison. The resulting AAI values were in the range of 51.7–60.8% (mean value 55.1 ± 1.1%), which correlates with the distribution of AAIs between different species of the same class (Konstantinidis and Tiedje, 2005).

The order *Thiohalospirales* includes a single family *Thiohalospiraceae* and genus *Thiohalospira*, currently comprising two species: the type species *Thiohalospira halophila* based on multiple extremely halophilic isolates from hypersaline habitats with neutral pH and an alkaliphilic, moderately halophilic species *Thiohalospira alkaliphila* from alkaline hypersaline lakes. Both species are sulfur-oxidizing, aerobic chemolithoautotrophs (Sorokin, 2008; Sorokin et al., 2006, 2008, 2020).

Type genus: **Thiohalospira** Sorokin et al. 2008^{VP}.

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FIGURE 1. Phylogenomic placement of the order *Thiohalospirales* based on concatenated partial amino acid sequences of 120 bacterial conserved proteins (Parks et al., 2018; taxonomic designations correspond with the Genome Taxonomy DataBase 207). The tree was built using the IQ-TREE 2 program (Minh et al., 2020) with ultrafast bootstrap approximation (Minh et al., 2013) as well as approximate likelihood-ratio test for branches (Anisimova and Gascuel, 2006). Bootstrap consensus tree is shown with values above 70% placed at the nodes. Bar, 0.10 changes per position.

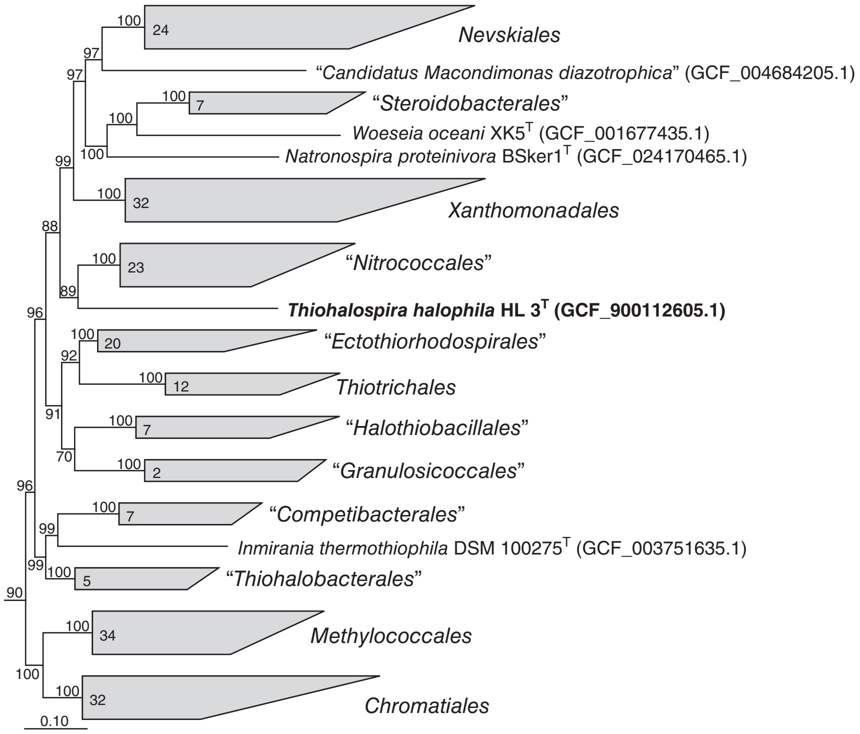

TABLE 1. The average amino acid identity (AAI) between the *Thiohalospira halophila H*L 3^T (GCF_900112605.1) genome and all available genomes of *Gammaproteobacteria* isolates using the EzAAI program (Kim et al., 2021)

Top hits in the *Gammaproteobacteria*	AAI (%)	Proteome coverage
Thiohalophilus thiocyanatoxydans DSM 16326 (GCF_004366735.1)	59.92	0.48
Thioalkalivibrio thiocyanodenitrificans ARhD 1 (GCF_000378965.1)	60.16	0.40
Inmirania thermothiophila DSM 100275 (GCF_003751635.1)	60.18	0.45
Ectothiorhodospira mobilis DSM 237 (GCF_016584115.1)	60.22	0.47
Sulfurivermis fontis JG42 (GCF_004001245.1)	60.34	0.45
Thioalbus denitrificans DSM 26407 (GCF_003337735.1)	60.42	0,39
Thiohalomonas denitrificans HLD2 (GCF_900102855.1)	60.57	0.44
Thiohalobacter sp. FOKN1 (GCF_002356355.1)	60.64	0.48
Thiohalobacter thiocyanaticus HRh1 (GCF_003932505.1)	60.78	0.44

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