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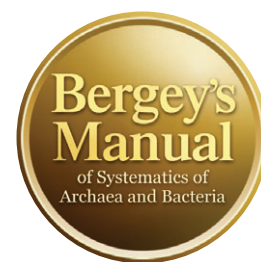
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Proteobacteria/Gammaproteobacteria/

Thiohalobacterales ord. nov.

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Thi.o.ha.lo.bac.ter.al'es N.L. masc. n. *Thiohalobacter*, the type genus of the order; L. fem. pl. n. suff. *-ales*, ending to denote an order; N.L. fem. pl. n. *Thiohalobacterales*, the order of the genus *Thiohalobacter*.

The order *Thiohalobacterales* accommodates obligately chemolithoautotrophic aerobic bacteria utilizing inorganic sulfur compounds as the energy source and assimilating CO₂ via the Calvin–Benson–Bassham cycle. The order is a deeply branching member of the *Gammaproteobacteria* and consists of two families *Thiohalobacteraceae* and *Thiogramaceae*, both of which include one genus. The order-level status was established by phylogenomic analysis based on the Genome Taxonomy DataBase classification.

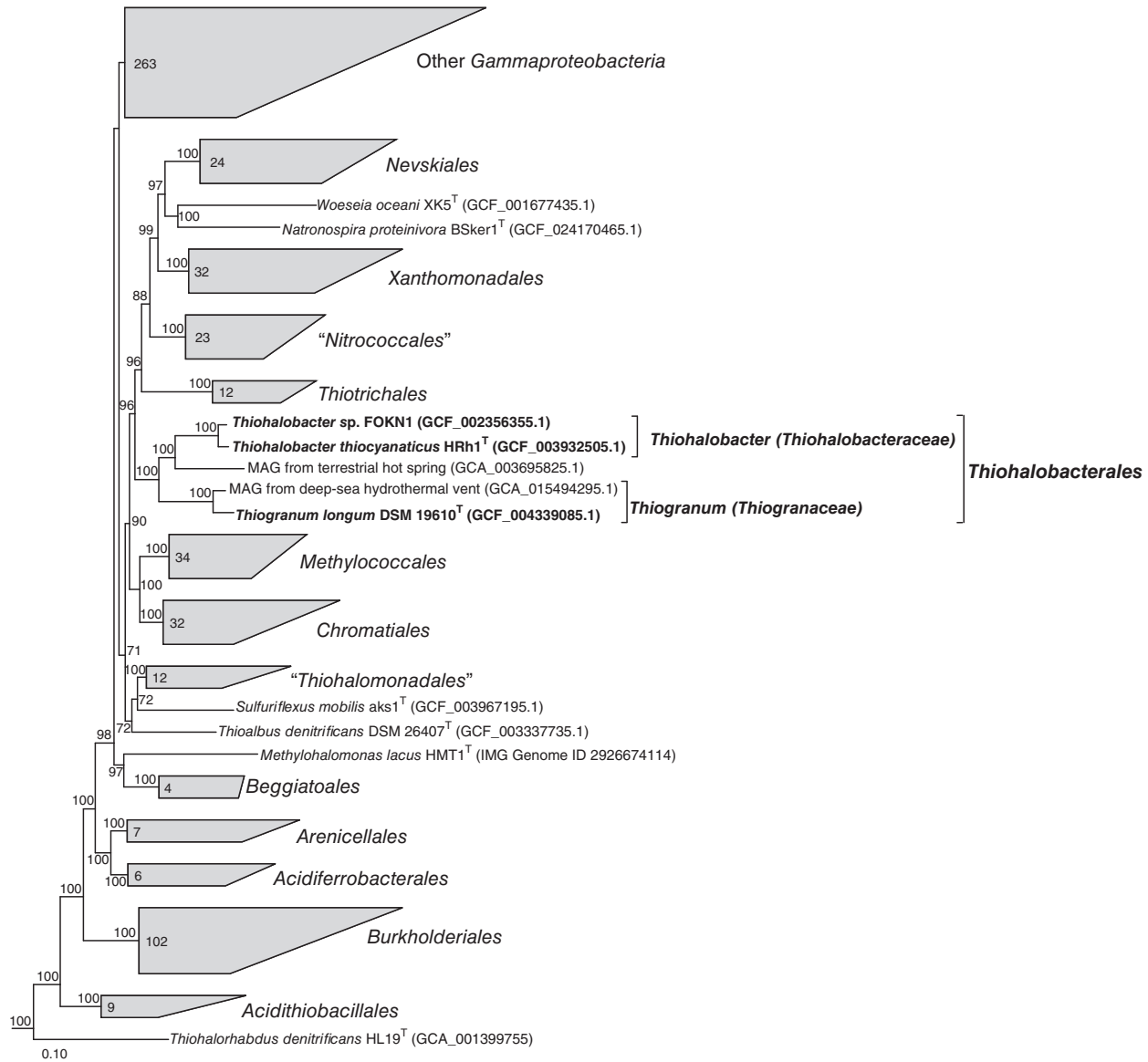
Type genus: Thiohalobacter Sorokin et al. 2010^{VP}.

In the original paper of Sorokin et al. (2010), the genus *Thiohalobacter* was described as an unaffiliated genus within the *Gammaproteobacteria*, and the genus *Thiogram* was classified in the order *Chromatiales* based on 16S rRNA sequence analyses (Mori et al., 2015). The use of phylogenomic

approach based on the analysis of 120 single copy conserved bacterial marker proteins revealed that the genera *Thiohalobacter* and *Thiogram* form a deeply branching lineage representing a separate order in the *Gammaproteobacteria* (Figure 1). The order *Thiohalobacterales* includes two families: *Thiohalobacteraceae* and *Thiogramaceae*, each of which comprises single monospecific genera, *Thiohalobacter* and *Thiogram*.

The average amino acid identity (AAI) between the two *Thiohalobacterales* representative genomes and all available genomes of the species in the *Gammaproteobacteria* isolates were calculated using 39–59% of all genes (1,400 on average). The resulting AAI values (the top list is shown in Table 1) were in the range of 51.4–62.9% (mean value 56.1 ± 1.3%), which correlates well with the distribution of AAI between different species of the same class (Konstantinidis and Tiedje, 2005). In such case, it is the phylogenetic reconstruction which should be used as decisive to define the taxonomy rank above the genus level. The AAI value between the two representative genera of this order, *Thiohalobacter* and *Thiogram*, was 63.4%.

FIGURE 1. Phylogenetic position of the order *Thiohalobacterales* based on sequence analyses of concatenated alignment of 120 single copy conserved bacterial protein markers (Parks et al., 2018; taxonomic designations correspond to the Genome Taxonomy DataBase) within the *Gammaproteobacteria*. The trees were built using the IQ-TREEs 2 program (Minh et al., 2020) with 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.



Both genera include obligately chemolithoautotrophic, sulfur-oxidizing bacteria (Sorokin et al., 2010; Mori et al., 2015) from saline habitats. The type genus of the *Thiohalobacterales* order is *Thiohalobacter*. It is a moderately halophilic bacterium with a unique capability to utilize thiocyanate as the energy and nitrogen source (Sorokin, 2008; Sorokin et al., 2006, 2010, 2020). The type strain of *Thiohalobac-*

ter thiocyanaticus was enriched from hypersaline lakes with neutral pH in southwestern Siberia. *Thiogranum longum* is a low salt-tolerant, obligately aerobic, chemolithotrophic sulfur-oxidizing bacterium from a marine habitat able to utilize elemental sulfur, thiosulfate, and sulfide as electron donors (Mori et al., 2015).

Type genus: Thiohalobacter Sorokin et al. 2010^{VP}.

TABLE 1. The average amino acid identity (AAI) between the *Thiohalobacteriales* representative genomes and the most related genomes in the *Gammaproteobacteria* using the EzAAI program (Kim et al., 2021)

<i>Thiohalobacteriales</i> representative	RefSeq assembly accession	Species	AAI (%)	Proteome coverage
<i>Thiogramum longum</i> DSM 19610 (GCF_004339085.1)	GCF_002000365.1	<i>Thioalkalivibrio denitrificans</i> ALJD	61.18	0.42
	GCF_900110965.1	<i>Ectothiorhodospira magna</i> B7-7	61.18	0.39
	GCF_000378965.1	<i>Thioalkalivibrio thiocyanodenitrificans</i> ARhD 1	61.20	0.41
	GCF_004366735.1	<i>Thiohalophilus thiocyanatoxydans</i> DSM 16326	61.25	0.50
	GCF_000021985.1	<i>Thioalkalivibrio sulfidiphilus</i> HL-EbGr7	61.36	0.45
	GCF_900102855.1	<i>Thiohalomonas denitrificans</i> HLD2	61.39	0.44
	GCF_000377945.1	<i>Thioalkalivibrio sulfidiphilus</i> ALJ17	61.69	0.46
	GCF_003337735.1	<i>Thioalbus denitrificans</i> DSM 26407	61.73	0.41
	GCF_004001245.1	<i>Sulfurivermis fontis</i> JG42	61.81	0.46
	GCF_003967195.1	<i>Sulfuriflexus mobilis</i> aks1	61.81	0.51
<i>Thiohalobacter thiocyanaticus</i> HRh1 (GCF_003932505.1)	GCF_003932505.1	<i>Thiohalobacter thiocyanaticus</i> HRh1	63.41	0.54
	GCF_900156225.1	<i>Ectothiorhodospira magna</i> B7-7	61.75	0.39
	GCF_900110965.1	<i>Ectothiorhodospira magna</i> B7-7	61.97	0.39
	GCF_002000365.1	<i>Thioalkalivibrio denitrificans</i> ALJD	61.97	0.45
	GCF_000378965.1	<i>Thioalkalivibrio thiocyanodenitrificans</i> ARhD 1	61.97	0.43
	GCF_000021985.1	<i>Thioalkalivibrio sulfidiphilus</i> HL-EbGr7	62.00	0.46
	GCF_900102855.1	<i>Thiohalomonas denitrificans</i> HLD2	62.05	0.46
	GCF_000377945.1	<i>Thioalkalivibrio sulfidiphilus</i> ALJ17	62.22	0.48
	GCF_013343005.1	<i>Thioalbus denitrificans</i> DSM 26407	62.57	0.42
	GCF_004001245.1	<i>Sulfurivermis fontis</i> JG42	62.81	0.46
GCF_004366735.1	<i>Thiohalophilus thiocyanatoxydans</i> DSM 16326	62.92	0.55	
GCF_004339085.1	<i>Thiogramum longum</i> DSM 19610	63.41	0.54	

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