# Genome-Based Phylogeny of the Genus *Thiothrix*

#### Subjects: Microbiology

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Representatives of the genus *Thiothrix* are filamentous, sulfur-oxidizing bacteria, capable of lithoauto-, lithoheteroand organoheterotrophic growth. They are often found in flowing waters with counter-oriented sulfide and oxygen gradients. They were first described at the end of the 19th century, but the first pure cultures of this species only became available 100 years later. An increase in the number of described *Thiothrix* species at the beginning of the 21st century shows that the classical phylogenetic marker, 16S rRNA gene, is not informative for species differentiation, which is possible based on genome analysis.

Thiothrix phylogeny pangenome metagenome-assembled genome

## 1. Introduction

The first attempts to describe bacteria from the genus *Thiothrix* were made by Rabenhorst as early as 1865, when he described the first member of this genus as *Beggiatoa nivea* <sup>[1]</sup>. Winogradsky, in turn, based on studies of key features of enrichment culture, named a new genus, *Thiothrix* <sup>[2]</sup>. The genus *Thiothrix* belongs to the class *Gammaproteobacteria*, order *Thiotrichales*, family *Thiotrichaceae*.

Habitats of *Thiothrix* vary from natural sulfidic waters, irrigation systems, and activated sludge in wastewater treatment plants to ectosymbionts of invertebrates in deep-sea hydrotherms. The influx of  $H_2S$  into the growth zone of these bacteria occurs from sulfidic springs, either from the near-bottom layers of sediments (in the shallow waters of lakes, in ponds, sea littorals, etc.) or from hydrothermal vents <sup>[3][4]</sup>. The hydrogen sulfide concentration can vary significantly—from tens of micrograms to several milligrams per litre. In nature, *Thiothrix* forms powerful foulings, visible to the naked eye.

Currently, the genus *Thiothrix* includes aerobic and facultative anaerobic, attached, filamentous, non-motile bacteria. They are capable of auto- and heterotrophic growth and are characterized by a respiratory type of metabolism. During autotrophic growth, CO<sub>2</sub> fixation occurs through the Calvin–Benson–Bassham cycle. Their ribulose-1,5-bisphosphate carboxylase-oxygenase (RuBisCO) belongs to types IAq, IAc, and II. All genomes contain genes encoding all enzymes of the Krebs cycle, with the exception of malate dehydrogenase (MDH), which is functionally replaced by malate:quinone oxidoreductase (MQO). *Thiothrix* spp. are capable of organotrophic growth in the presence of reduced sulfur compounds. During lithotrophic growth in the presence of reduced sulfur compounds. During lithotrophic growth in the presence of networks accumulated intracellularly. Hydrogen sulfide

is oxidized to sulfur by the sulfide:quinone oxidoreductase (SQR) and flavocytochrome *c*-sulfide dehydrogenase (FCSD). Thiosulfate is oxidized by the branched sulfur-oxidizing system (SOX) pathway without SoxCD with the formation of sulfur and sulfate. Sulfite is oxidized via direct (membrane-bound cytoplasmic sulfite:quinone oxidoreductase (SoeABC) and indirect (adenosine phosphosulfate reductase (AprAB) and ATP sulfurylase (Sat)) oxidation pathways (**Figure 1**).



**Figure 1.** Scheme of dissimilation sulfur metabolism of the genus *Thiothrix*. FccAB, flavocytochrome *c*-sulfide dehydrogenase; SqrA/F, sulfide:quinone oxidoreductase; SoxAXBYZ, SOX multienzyme system; MQ, menaquinone; rDsrABCEFHEMPKJOL, dissimilatory sulfite reductase; AprABM, APS reductase; Sat, ATP sulfurylase; SoeABC, membrane-bound cytoplasmic sulfite:quinone oxidoreductase; S<sup>0</sup>, sulfur globule; APS, adenosine 5'-phosphosulfate;  $R-S_{n+1}H$ , thiol compound;  $H-S_{n+1}$ , polysulfide.

Before 1965, eleven morphotypes of the genus *Thiothrix* were described mainly in natural marine and freshwater habitats containing hydrogen sulfide <sup>[5][6][7]</sup>. These microorganisms are differentiated based solely on the diameter of the filaments and the characteristics of the habitat. Subsequently, obtaining pure cultures made it possible to reveal that the morphology of the genus *Thiothrix* is variable <sup>[8]</sup>. The final invalidity of using phenotypic characters for the taxonomy of the genus *Thiothrix* was confirmed by Howarth et al., 1999 <sup>[9]</sup>. In 1983, Larkin and Shinabarger isolated the first pure culture for a representative of this genus <sup>[5]</sup>. Based on Winogradsky's description, Shinabarger suggested that the culture he received was *Thiothrix nivea*. The strain JP2 with a validly published name *Thiothrix nivea* JP2<sup>T</sup> (=ATCC 35100<sup>T</sup> = DSM 5205<sup>T</sup>) is the only established neotype of the species <sup>[5]</sup>.

At the end of the 20th century, several new isolates were obtained: *Thiothrix ramosa* <sup>[10]</sup>, *Thiothrix arctophila* <sup>[11]</sup>, and *Thiothrix* sp. CT3 <sup>[12]</sup>. Unfortunately, two proposed species, *T. ramosa* <sup>[10]</sup> and *T. arctophila* <sup>[11]</sup>, are absent in international collections and were lost (Dubinina, personal communication).

The genus *Thiothrix* was significantly expanded by Howarth in 1999 <sup>[9]</sup>. Four new species were included in the genus: *Thiothrix fructosivorans*, *Thiothrix unzii*, *Thiothrix defluvii*, and *Thiothrix eikelboomii*. The last two species were assigned to the Eikelboom type 021N group within the genus *Thiothrix*, and the species *T. nivea*, *T. fructosivorans*, and *T. unzii* were assigned to the *T. nivea* group <sup>[9]</sup>. Comparative analysis of the 16S rRNA gene sequence of members of the Eikelboom type 021N and the *T. nivea* groups showed low similarity (90–91%). However, the notable phenotypic similarity between the Eikelboom type 021N group and the *Thiothrix nivea* group did not allow division into new genera at that time.

Two extra representatives of Eikelboom type 021N, *Thiothrix disciformis* and *Thiothrix flexilis*, and two species from the *Thiothrix nivea* group, *Thiothrix lacustris* and *Thiothrix caldifontis*, were described in later years <sup>[13][14]</sup>.

The increase in the number of species of the genus *Thiothrix* has set the task of searching for new phylogenetic markers. Pure cultures of the genus *Thiothrix* isolated from various biotopes (hydrogen sulfide springs, wastewater treatment plants, the White Sea littoral, activated sludge treatment systems, freshwater lakes, groundwater, invertebrate ectosymbionts, etc.) have a similar morphotype, but a rather variable metabolism. The phylogeny based on the 16S rRNA gene sequences does not always correspond to the phylogenetic diversity of the representatives of this group <sup>[8]</sup>. Some strains assigned to the same species based on the 16S rRNA gene were reclassified as separate species after determination of whole-genome sequences (*T. lacustris* BL<sup>T</sup>, '*Thiothrix litoralis*' AS<sup>T</sup>, and '*Thiothrix winogradskyi*' CT3<sup>T</sup>) (**Figure 2**).

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		Genome	AS <sup>T</sup>	$BL^{T}$	$\mathbf{Q}^{\mathrm{T}}$	$G1^{T}$	CT3 <sup>T</sup>	KT	Ku-5 <sup>T</sup>	$A1^{T}$	A52	207	RT	JP2 <sup>T</sup>	SSD2	16S rRNA, %
	[	AST	100.00	100.00	98.81	98.74	98.81	98.81	98.81	94.91	94.91	—	95.39	95.10	94.42	100
	٦L	$BL^{T}$	91.10	100.00	98.88	98.81	98.88	98.88	98.88	94.98	94.98	—	95.46	95.17	94.49	78
		$\mathbf{Q}^{\mathrm{T}}$	80.80	80.42	100.00	99.16	99.37	99.72	99.86	95.32	95.32	—	95.25	94.67	94.00	72
		$G1^{T}$	79.85	79.26	81.18	100.00	98.81	99.16	99.16	95.05	95.05	—	95.67	95.23	94.14	
		CT3 <sup>T</sup>	79.79	79.18	81.43	84.01	100.00	99.23	99.37	95.18	95.19	—	95.32	94.54	94.15	
	11	KT	79.82	79.32	81.50	84.03	86.37	100.00	99.72	95.46	95.46	—	95.25	94.60	93.93	
	L	Ku-5 <sup>T</sup>	80.20	79.42	82.24	85.34	87.96	91.00	100.00	95.32	95.32	—	95.25	94.67	94.00	
		A1 <sup>T</sup>	76.94	76.32	78.54	78.81	78.94	78.23	79.39	100.00	100.00	—	96.23	94.84	94.37	
ANI, %	L	A52	76.27	76.08	77.19	78.32	79.70	78.13	78.86	89.62	100.00	—	96.23	94.70	94.37	
100	ſ	207	77.40	76.62	75.90	76.00	75.28	75.07	76.16	75.72	75.03	100.00	—	—	—	
78	Γ	RT	76.87	76.19	76.49	76.93	77.50	76.17	76.68	76.71	76.63	79.13	100.00	97.27	96.17	
72	Г	$JP2^{T}$	77.26	76.39	75.75	75.59	75.74	75.59	75.83	74.24	74.06	79.40	77.53	100.00	96.16	
12	1	SSD2	75.97	75.49	74.61	74.81	74.81	74.66	74.73	73.57	73.06	77.71	76.17	79.88	100.00	

**Figure 2.** Heatmap of 16S rRNA gene sequence similarity and pairwise ANI values (%) for *Thiothrix* genomes. *T. lacustris* BL<sup>T</sup>, (GCF\_000621325.1); *Thiothrix litoralis* AS<sup>T</sup> (GCF\_017901135.1); *'Thiothrix subterranea'* Ku-5<sup>T</sup> (GCF\_016772315.1); *'Ca.* Thiothrix sulfatifontis' KT (GCA\_022828425.1); *T. caldifontis* G1<sup>T</sup> (GCF\_900107695.1);

'*Thiothrix winogradskyi*'  $CT3^{T}$  (GCF\_021650945.1); *T. fructosivorans*  $Q^{T}$  (GCA\_017349355.1); *T. unzii*  $A1^{T}$  (GCA\_017901175.1); '*Ca.* Thiothrix anitrata' A52 (GCF\_017901155.1); *Ca.* Thiothrix moscovensis RT (GCA\_016292235.1); *T. nivea* JP2<sup>T</sup> (GCF\_000260135.1); *Ca.* Thiothrix singaporensis SSD2 (GCA\_013693955.1); MAG of *Thiothrix* sp. 207 (GCA\_018813855.1). Note that 16S rRNA gene is missing in MAG of *Thiothrix* sp. 207.

However, the 16S rRNA gene can be successfully used to identify *Thiothrix* at the genus level since the levels of 16S rRNA gene sequence identity between representatives of the genus *Thiothrix* exceed 94%, while with members of other genera, this value is below 91%.

### 2. Genome-Based Phylogeny

The determination of complete genome sequences for *T. disciformis*, *T. eikelboomii*, *T. flexilis*, *T. caldifontis*, *T. lacustris*, and *T. nivea* has enabled a more accurate phylogenetic analysis. In 2018, Boden and Scott undertook a multi-phase study which included morphological, biochemical, physiological, and genomic properties, and genebased phylogeny to reclassify *Thiothrix* species. The 16S rRNA gene (*rrs*), recombination protein A (*recA*), polynucleotide nucleotide transferase (*pnp*), translation initiation factor IF-2 (*infB*), glyceraldehyde-3-phosphate dehydrogenase (*gapA*), glutamyl-tRNA synthetase (*glnS*), elongation factor EF-G (*fusA*), and concatenated sequences of 53 ribosomal proteins allowed the distribution of *Thiothrix* species between three different families: *Thiolineaceae*, *Thiofilaceae*, and *Thiotrichaceae* <sup>[15]</sup>.

*Thiothrix defluvii* and *Thiothrix flexilis* were reclassified as representatives of the new genus *Thiofilum* within the family *Thiofilaceae* with the proposed names *Thiofilum flexile* and *Thiofilum defluvii*. *Thiothrix eikelboomii* and *Thiothrix disciformis* were placed in the new genus *Thiolinea* within the new family *Thiolineaceae* with the proposed names *'Thiolinea eikelboomii*' for *Thiothrix eikelboomii*. However, the reclassification of *Thiolinea eikelboomii* is currently only formal due to the lack of cultures in two international collections, as required for species validation. *T. caldifontis*, *T. lacustris*, *T. nivea*, *T. unzii*, and *T. fructosivorans* remained in the genus *Thiothrix* <sup>[15]</sup>.

The development of genomics and metagenomics methods made it possible to obtain complete genome sequences and use them for phylogenetic studies, which, in turn, contributed to the development of a new genome-based taxonomic system of prokaryotes <sup>[16]</sup>. The whole-genome comparison has higher accuracy and resolution than taxonomy based on individual phylogenetic markers. Whole-genome sequences of isolates *T. fructosivorans*  $Q^T$ , *T. unzii*  $A1^T$ , *Thiothrix litoralis*  $AS^T$ , '*Thiothrix subterranea*' Ku- $5^T$  <sup>[8]</sup>, and '*Thiothrix winogradskyi*' CT3<sup>T</sup> <sup>[17]</sup>, as well as metagenome-assembled genomes (MAGs) of '*Candidatus* Thiothrix anitrata' A52, *Candidatus* Thiothrix moscovensis RT <sup>[18][19]</sup>, and *Candidatus* Thiothrix sulfatifontis' KT was obtained from the fouling of a hydrogen sulfide source <sup>[17]</sup>.

The main characteristics of the obtained genomes are shown in Table 1.

**Table 1.** The general properties of *Thiothrix* genomes.

Species	Genome Assembly	Isolate or MAG	Size (MB)	Contigs	G + C I Content (mol %)	Protein- Coding Genes	16S rRNA Genes	tRNA F Genes	Plasmids *
<sup>'</sup> T. winogradskyi' CT3 <sup>⊤</sup> (DSM 12730 <sup>⊤</sup> )	GCA_021650935.1	Isolate	4.38	3	51.4	4292	5	66	2
'Ca. Thiothrix sulfatifontis' KT	GCA_022828425.1	MAG	3.69	1	51.5	3729	2	47	NA
<i>T. lacustris</i> BL <sup>⊤</sup> (DSM 21227 <sup>⊤</sup> )	GCF_000621325.1	Isolate	3.72	56	51.3	3537	2	40	U
<i>T. litoralis</i> AS <sup>⊤</sup> (DSM 113264 <sup>⊤</sup> )	GCF_017901135.1	Isolate	4.28	1	52.8	4045	3	44	0
<sup>(</sup> T. subterranea' Ku-5 <sup>T</sup> (VKM B-3544 <sup>T</sup> )	GCF_016772315.1	Isolate	4.08	4	51.1	3885	4	46	3
T. caldifontis G1 <sup>⊤</sup> (DSM 21228 <sup>⊤</sup> )	GCF_900107695.1	Isolate	3.94	72	50.6	3752	1	42	U
<i>T. unzii</i> A1 <sup>⊤</sup> (ATCC 49747 <sup>⊤</sup> )	GCA_017901175.1	Isolate	3.72	8	50.8	3626	2	45	7
<i>T. nivea</i> JP2 <sup>T</sup> (DSM 5205 <sup>T</sup> )	GCF_000260135.1	Isolate	4.69	15	54.9	4327	2	44	U
T. fructosivorans Q <sup>T</sup> (ATCC 49748 <sup>T</sup> )	GCA_017349355.1	Isolate	4.36	6	51.3	3616	2	44	5
<i>Ca.</i> Thiothrix moscovensis RT	GCA_016292235.1	MAG	3.69	78	53.6	3483	1	38	NA
<i>Ca.</i> Thiothrix singaporensis SSD2	GCA_013693955.1	MAG	4.54	1	55.6	4097	2	43	NA

Species	Genome Assembly	Isolate or MAG	Size (MB)	Contigs	G + C Content (mol %)	Protein- Coding Genes	16S rRNA Genes	tRNA Genes	Plasmids *	en. When
'Ca. Thiothrix anitrata' A52	GCF_017901155.1	MAG	3.55	1	50.1	3387	2	45	NA	313855.1, ween the
Thiothrix sp. 207 207, was choser	GCA_018813855.1	MAG	3.93 een ivi/	136 AG 01 771	54.6 IOUTITIX SD.	3675 207 anu	0 other r	40 epresen	NA tatives of	r analysis othrix sp. the genus
(76–79% and 20 * U, un <b>2</b> ).	–26%, respectively) known because the	indicated assembly	that t consis	his geno sted of m	me repres ultiple cor	sented a ntigs; NA,	novel ( not ap	Candida blicable	<i>tus</i> specie for MAGs.	s ( <b>Figure</b>

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