Reclassification of *Thi microspira hydrogeniphila* (Watsuji et al. 2016) to *Thi microrhabdus hydrogenophila* comb. nov., with emended description of *Thi microrhabdus* (Boden et al., 2017)

Rich Boden,¹² Kathleen M. Scott,³ Alexander W. Rae¹ and Lee P. Hutt¹²

**Abstract**

The genus *Thi microrhabdus* (Tmr) in the Piskirickettsiaceae in the Thirichiales of the Gammaproteobacteria contains four species of sulfur-oxidising obligate chemolithoautotroph with validly published names, all previously classified as *Thi microsoria* (Tms) species. Here we demonstrate that *Thi microsoria hydrogeniphila*, a recently published hydrogen-utilising chemolithoautotroph closely related to *Thi microrhabdus frisia* (type species of *Thi microrhabdus*) should be classified as a member of the genus *Thi microrhabdus* and not *Thi microsoria*, as *Thi microrhabdus hydrogeniphila* comb. nov., on the basis of comparative physiological and morphological research as well as 16S rRNA (rRNA) gene identity of *Tms. hydrogeniphila* MAS² being closer to that of *Tmr. frisia* JB-A2¹ (99.1 %) than to *Tms. pelophila DSM 1534³ (90.5 %) or *Hydrogenovibrio marinus* MH-110⁴ (94.1 %), and on the basis of the topology of 16S rRNA gene maximum likelihood trees, which clearly place *Tms. hydrogeniphila* within the genus *Thi microrhabdus*. It was also noted that thiosulfate-grown *Thi microrhabdus* spp. can be distinguished from *Thi microrhabdus* spp. or *Hydrogenovibrio* spp. on the basis of the 3 dominant fatty acids (C₁₆:₁, C₁₈:₁, and C₁₆:₀), and from other *Thi microrhabdus* spp. on the basis of the fourth dominant fatty acid, which varies between the species of this genus – which could provide a useful diagnostic method. We provide an emended description of *Thi microrhabdus* (Boden R, Scott KM, Williams J, Russel S, Antonen K et al. *Int J Syst Evol Microbiol* 2017;67:1140–1151) to take into account the properties of *Thi microrhabdus hydrogeniphila* comb. nov.

The taxonomy and systematics of the genera *Thi microsoria* (Tms), *Hydrogenovibrio* (H) and *Thioalkalimicrobium* (Tam) were recently revised [1], proposing the genus *Thi microrhabdus* (Tmr) for a clade of rod-shaped organisms previously classified as *Thi microsoria* spp., and reclassifying one other clade of *Thi microsoria* spp. as *Hydrogenovibrio* spp., leaving only *Tms. pelophila* (type species) and *Tms. thyasirae* as *Thi microsoria* spp., but all four members of *Thioalkalimicrobium* were also reclassified to *Thi microsoria*. This was based on a polyphasic study of chemotaxonomic, physiological, genomic and phylogenetic elements. Shortly before our study [1] was accepted, *Thi microsoria hydrogeniphila* [2] was published, based on a eurypsychrophilic hydrogen and sulfur-oxidising chemolithoautotroph isolated from enrichment cultures inoculated with the slurry of a 10-month-old tank of seawater from Toyko Bay, Japan, that had been incubated with a block of beef tallow at 10 °C. Here we present the short case that this species should also be circumscribed into the genus *Thi microrhabdus* per all other members of the same clade of *Thi microsoria* as proposed by Boden et al. [1], as *Thi microrhabdus hydrogeniphila* comb. nov., and we accordingly revise the description of *Thi microrhabdus* [1] to take into account the properties of this species.

*Thi microrhabdus* [1] comprises four species with validly published names – *Thi microrhabdus frisia* [3], *Thi microrhabdus chilensis* [4], *Thi microrhabdus arctica* [5] and *Thi microrhabdus psychrophila* [5]. All were isolated from marine sediments and can use thiosulfate and other reduced sulfur species as electron donors but none have been found to use molecular hydrogen thus far. *Tmr. psychrophila* and *Tmr. arctica* are stenopsychrophilic, whereas the other two species are eurypsychrophilic and have more mesophilic temperature optima. All species are moderate halophiles requiring 40–100 mM sodium chloride (2.3–5.8 g l⁻¹) for growth, with optima of 250–400 mM (14.6–23.4 g l⁻¹). *Thi microrhabdus* spp. share the same three dominant fatty acids [palmitoleic (C₁₆:₁), vaccenic (C₁₈:₁), palmitic (C₁₆:₀) acids] when grown on thiosulfate agar plates, but can be easily distinguished by their next-dominant fatty acid [Tmr. *chilensis*, stearic acid (C₁₈:₀); Tmr. *arctica*, myristoleic acid.

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**Keywords:** chemolithoautotroph; Thi microspira; Thi microrhabdus; Hydrogenovibrio; Thioalkalimicrobium; hydrogen.

**Abbreviations:** Tam, Thioalkalimicrobium; Tmr, Thi microrhabdus; Tms, Thi microspira.
H. marinus. to (on the basis of the proposed genus cut-off of 94.5% identity is not the same genus as either species) of 99.1% versus 90.5% to Thiomicrorhabdus genus be seen from Fig. 1 that 16S rRNA gene sequences of this clade of organisms were curated for phylogenetic analyses, but are confident from our previous study of this group of genera that this gene indeed accurately reflects the phylogeny using more genes. Gene sequences of this clade of organisms were curated from the GenBank database and aligned using the MUSCLE algorithm [7] in MEGA 7.0.2 [8]. A maximum-likelihood tree (Fig. 1) was built using the Tamura-Nei model [9]. Bootstrap values at nodes represent 5000 resamplings of the tree, and are shown where greater than or equal to 70%. It can be seen from Fig. 1 that Tms. hydrogeniphila falls within the genus Thiomicrorhabdus, which is further supported on the basis of the 16S rRNA gene identity to Tmr. frisia (type species) of 99.1%, versus 90.5% to Tms. pelophila and 94.1% to H. marinus. These data indicate that Tms. hydrogeniphila is not the same genus as either Tms. pelophila or H. marinus on the basis of the proposed genus cut-off of 94.5% identity (‘the Yarza cut-off’ [10]), and on the basis of tree topology.

It is worth noting that Tms. hydrogeniphilus was robustly demonstrated by Watsuji et al. [2] to be distinct from Tmr. frisia on the basis of DNA–DNA hybridisation, thus we regard it as a distinct species. We have also previously demonstrated [1] that the other species of Thiomicrorhabdus (and Hydrogenovibrio and Thiomicrospira) are also correctly circumscribed on the basis of in silico DNA–DNA hybridisation scores.

Differential properties of Tms. hydrogeniphila versus Thiomicrospira sensu stricto, Hydrogenovibrio and Thiomicrorhabdus spp. are curated in Table 1. It can be seen that the cell size, morphology, maximum specific growth rate on thiosulfate, motility and flagellation are more similar to Thiomicrorhabdus spp. rather than to Thiomicrospira spp. or Hydrogenovibrio spp. The temperature range and optimum for Tms. hydrogeniphila are very similar to the nearest neighbour in terms of the 16S rRNA gene, Thiomicrorhabdus frisia (type species). The dominant three fatty acids of Tms. hydrogeniphila are identical to all Thiomicrorhabdus spp. but fall in a different order to Thiomicrospira and Hydrogenovibrio spp. The fourth-dominant fatty acid is lauric acid (C12:0), which is distinct from the other Thiomicrorhabdus spp. and could provide a means to diagnose speciation with rapidity since all species differ in this fourth

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**Fig. 1.** Maximum likelihood tree based on the 16S rRNA (rrs) gene sequences from Thiomicrospira hydrogeniphila MAS2(T) and all members of the genera Thiomicrospira, Thiomicrorhabdus, Galenea and Hydrogenovibrio with validly published names. The outgroup is the gene sequence from Halothiobacillus neapolitanus NCIMB 8539(T). Genes were aligned using the MUSCLE algorithm in MEGA 7.0.20 and trees built using the Tamura-Nei model with the nearest-neighbour interchange (NNI) heuristic method and partial deletion of gaps. Topology with the superior log-likelihood is shown, with numbers at nodes representing the percentage of 5000 bootstrap replicates for which that topology was preserved (values <70% are omitted). GenBank gene accession numbers are giving in parentheses. Scale bar represents the number of substitutions per site. 1270 nt were used in the analysis. Type species of genera are emboldened and the test sequence (Tms. hydrogeniphila MAS2(T)) is underlined.
Table 1. Comparative properties of *Thiomicrospira hydrogeniphila* MAS2<sup>T</sup> with all species of *Thiomicroarhabdus* with validly published names, and conglomerated properties of *Thiomicrospira* and *Hydrogenovibrio*

Data are from [1] or [2]. Values are positive (+), weak positive (±), negative (−) or not determined (ND).

<table>
<thead>
<tr>
<th></th>
<th><em>Thiomicroarhabdus hydrogeniphila</em> MAS2&lt;sup&gt;T&lt;/sup&gt;</th>
<th><em>Thiomicroarhabdus frisia</em> JB-A2&lt;sup&gt;T&lt;/sup&gt;</th>
<th><em>Thiomicroarhabdus chilensis</em> Ch-1&lt;sup&gt;T&lt;/sup&gt;</th>
<th><em>Thiomicroarhabdus arctica</em> SVAL-E&lt;sup&gt;T&lt;/sup&gt;</th>
<th><em>Thiomicroarhabdus psychrophila</em> SVAL-D&lt;sup&gt;T&lt;/sup&gt;</th>
<th><em>Thiomicrospira</em> spp.</th>
<th><em>Hydrogenovibrio</em> spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>16S rRNA gene sequence identity to (%):</strong></td>
<td>90.5 91.6 92.0 92.9 92.9 95.9</td>
<td>99.1 100.0 96.0 96.0 96.0</td>
<td>94.1</td>
<td>94.2</td>
<td>94.8</td>
<td>94.0</td>
<td>94.1</td>
</tr>
<tr>
<td><strong>Properties</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Colony colour on thiosulfate agar grown under air</td>
<td>White, cream</td>
<td>White, yellow</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>White, pink or red</td>
<td>White, cream or yellow ±</td>
</tr>
<tr>
<td>Heterotrophic</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>+G+C fraction (mol%)</td>
<td>39.6 39.6 49.9 42.4 42.5 45.6</td>
<td>99.1 100.0 96.0 96.0 96.0</td>
<td>93.3</td>
<td>93.3</td>
<td>93.3</td>
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<td>93.3</td>
</tr>
<tr>
<td>Maximum specific growth rate on thiosulfate under optimal conditions (h&lt;sup&gt;−1&lt;/sup&gt;)</td>
<td>0.4 0.45 0.4</td>
<td>0.4</td>
<td>0.14</td>
<td>0.2</td>
<td>0.07–0.33</td>
<td>0.25–0.8</td>
<td></td>
</tr>
<tr>
<td>Temperature range (°C)</td>
<td>20.0–40.0 3.5–39.0</td>
<td>3.5–42.0</td>
<td>–2.0–20.8</td>
<td>–2.0–20.8</td>
<td>3.5–41.0</td>
<td>3.5–55.0</td>
<td></td>
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<tr>
<td>Dominant fatty acids in thiosulfate-grown cells</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;:1</td>
<td>ND</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;:1</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;:1</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;:1</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;:1</td>
<td>C&lt;sub&gt;16&lt;/sub&gt;:1</td>
</tr>
<tr>
<td>Molecular hydrogen as an electron donor</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cell length (µm)</td>
<td>0.9–1.8</td>
<td>1.0–2.7</td>
<td>0.8–2.0</td>
<td>1.2–1.5</td>
<td>1.3–1.7</td>
<td>0.8–5.0</td>
<td>0.8–3.0</td>
</tr>
<tr>
<td>Cell width (µm)</td>
<td>0.3–0.5</td>
<td>0.3–0.5</td>
<td>0.3–0.5</td>
<td>0.5–0.6</td>
<td>0.5–0.6</td>
<td>0.2–2.0</td>
<td>0.2–0.7</td>
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<tr>
<td>Morphology</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>±</td>
<td>+</td>
<td>±</td>
<td>+</td>
</tr>
<tr>
<td>Flagella</td>
<td>1</td>
<td>ND</td>
<td>ND</td>
<td>1</td>
<td>1</td>
<td>0–3</td>
<td>1</td>
</tr>
</tbody>
</table>
dominant acid. The G+C fraction of genomic DNA is identical to *Tmr. frisia*, but as already stated, it has been distinguished from this species on the basis of DNA–DNA hybridisation previously, however, future *in silico* DNA–DNA hybridisation studies once the genome sequence is complete will be needed to confirm this since both the G+C fraction and 16S rRNA gene identity with *Tmr. frisia* are very high, yet the DNA–DNA hybridisation percentage of Watsuij et al. [2] is still below 20%, which appears rather low. *Tms. hydrogeniphila* can use molecular hydrogen as an electron donor, which is distinct from all *Thiomicroirhabdus* spp., and this warrants the emending of the genus description to add this property, which is found in some *Hydrogenovibrio* spp., but not in this genus.

We conclude that *Thiomicrospira hydrogeniphila* actually represents a member of the recently defined genus *Thiomicroirhabdus* (composed entirely of former *Thiomicrospira* spp.) and propose the name *Thiomicroirhabdus hydrogeniphila* comb. nov. to reflect this. We also propose some minor revisions to the Boden et al. [1] description of *Thiomicroirhabdus* to take into account the properties of *Tmr. hydrogeniphila* comb. nov. and other data from Watsuij et al. [2].

**EMENDED DESCRIPTION OF THIOMICRORHABDUS (BODEN ET AL. 2017)**

*Thiomicroirhabdus* (Thi.o.mi.cro.rhab’dus. Gr. n. theion, L. transliteration *thium*, sulfur; Gr. adj. mikrós, small; Gr. fem. n. rhabdos, N.L. transliteration *rhabdus*, rod or wand. N.L. fem. n. *Thiomicrospira*, small sulfur-oxidising rod).

Gram-stain-negative. Cells grown in liquid media are rod-shaped and motile by means of a single polar flagellum. Typical cell lengths are 0.8–2.7 µm and diameters are 0.3–0.6 µm, wider than *Thiomicrospira* spp. Do not form endospores or exospores. Use molecular oxygen as the sole terminal electron acceptor. Have a *cbb*₃-type cytochrome *c* oxidase (EC 1.9.3.1). Form white to yellow, entire colonies on thiosulfate-agar, coated in small granules of elemental sulfur. Motile, cells are monotrichous when grown in liquid media. Obligately chemolithoautotrophic with heterotrophy never observed. Can use thiosulfate, tetrathionate or sulfide as sole electron donors and at least one species uses molecular hydrogen, but none are known to use thiocyanate, sulfite, iron or manganese. Some species can use elemental sulfur as a sole electron donors. Fix carbon dioxide via the transaldolase-variant Calvin-Benson-Bassham cycle. All species use ammonium as a nitrogen source. Does not fix dinitrogen. No nitrogenase genes observed in genome sequences. Has form IAc and/or form IAq, and form II RuBisCo.

All species produce elementary sulfur when growing on thiosulfate at neutrality, but at varying degrees. Never axotrophic for vitamin B₁₂. Growth occurs from pH 4.2 to pH 9.0 but range varies with species – pH optima are pH 6.5 to 8.5. Grows from −2 to 42 °C with optima of 11.5 to 35 °C, varying by species, most of which are stenopsychrophilic or eurypsychrophilic. NaCl is required for growth, with minima of 40–100 mM, maxima of 1240 mM across the genus and optima of 250–470 mM. Does not reduce nitrate to nitrite. G+C fractions of genomic DNA are 39.6–49.9 mol%. Dominant respiratory quinone is ubiquinone-8. Dominant fatty acids are palmitoleic (C₁₆:1), vaccenic (C₁₈:1) and palmitic (C₁₆:0) acids, in that order, which is distinct from *Thiomicrospira* spp. and *Hydrogenovibrio* spp., but consistent with other *Thiomicroirhabdus* spp., followed by stearic (C₁₈:0) and myristoleic (C₁₄:1) acids. Members of the *Piskirickettsiaceae* in the *Thiotrichales* of the Gammaproteobacteria.

Type species: *Thiomicroirhabdus frisia* (Basonym: *Thiomicrospira frisia*) [3].

**DESCRIPTION OF THIOMICRORHABDUS HYDROGENIPHILA COMB. NOV.**

*Thiomicroirhabdus hydrogeniphila* (hy.dro.ge.ni’phi.la. Gr. n. hydor, water; Gr. v. gennaō, to beget, to bring forth, to produce; N.L. n. hydrogenum, hydrogen, i.e. that which produces water; N.L. adj. philus from Gr. adj. philos, friend, someone dearly loved; N.L. fem. adj. *hydrogeniphila*, hydrogen-loving).

Gram-stain-negative. Cells grown in liquid media are rod-shaped and motile by means of a single polar flagellum. Cells are 0.9–1.8 µm in length and 0.3–0.5 µm in diameter. Cells grown in MMJS broth [2] were obligately aerobic, using only molecular oxygen as their terminal electron acceptor and tolerating up to 40% (v/v) oxygen in a gas phase of nitrogen supplemented with 5% (v/v) carbon dioxide as sole carbon source, at atmospheric pressure. Nitrate, nitrite, ferric iron, ferrihydrite, selenate and fumarate are not used as terminal electron acceptors. Obligately chemolithoautotrophic, electron donors supporting growth are: thiosulfate, tetrathionate, elemental sulfur, sulfide and molecular hydrogen. Sulfite was not used. Oxidation of molecular hydrogen is repressed at high oxygen partial pressures. Ammonium was the sole nitrogen source, and nitrate, nitrite and molecular nitrogen were not used. Selenate, tungstate and vitamins (viz. biotin, folate, pyridoxine, thiamine, riboflavin, nicotinate, pantothenate, cyanocobalamin, p-aminobenzoate or lipoate) were not required for growth. Growth occurs between 2 and 40 °C with an optimum of 30 °C, and between pH 5.0 and pH 8.0 with an optimum of pH 6.0, in MMJS medium incubated under air. Growth occurs from 30 to 1380 mM Na⁺, with optimal growth at 270 mM Na⁺. Sensitive to ampicillin (50 µg ml⁻¹, 143 µM), chloramphenicol (50 µg ml⁻¹, 155 µM), kanamycin A (50 µg ml⁻¹, 103 µM), rifampicin (50 µg ml⁻¹, 60 µM), streptomycin (50 µg ml⁻¹, 86 µM); resistant to vancomycin (50 µg ml⁻¹, 35 µM). Heterotrophic or chemolithoheterotrophic growth (the latter with thiosulfate) is not observed on any carbon source tested, viz. yeast extract, peptone, tryptone, casein, starch, carboxymethylcellulose or casamino acids [each at 0.1% (w/v)]; formate, acetate, glycerol, citrate, tartarate, fumarate, malate, succinate,
propionate, lactate, oxalate, pyruvate or the 20 structural amino acids (each at 5 mM); glucose, galactose, fructose (each at 1.1 mM); sucrose, lactose, maltose or trehalose (each at 0.6 mM). Maximum specific growth rate on thiosulfate under optimal conditions was 0.4 h⁻¹. Dominant fatty acids in thiosulfate-grown cells are palmitic acid (C₁₆:0), palmitoleic acid (C₁₆:1) and vaccenic acid (C₁₈:1). Of the hydroxylated fatty acids, only 3-hydroxycaprylic fatty acids in thiosulfate-grown cells are palmitic acid (C₁₆:0), palmitoleic acid (C₁₆:1) and vaccenic acid (C₁₈:1).

Of the hydroxylated fatty acids, only 3-hydroxycaprylic acid (C₁₀:0 3-OH) is found. Lauroleic (C₁₂:1) and myristoleic (C₁₄:1) acids are not found, but lauric (C₁₂:0) and myristic (C₁₄:0) acids are in minor amounts – these four acids are diagnostic versus other Thiomicrorhabdus spp. (cf. Table 1). The G+C content of genomic DNA of the type strain is 39.6 mol% (HPLC).


Type strain is MAS2T=JCM 30760T=DSM 100274T, isolated from enrichment cultures using molecular hydrogen as the sole energy source, inoculated with slurry obtained from a sulfidic tank containing surface seawater from Tokyo Bay, supplemented with a block of beef tallow (c. 350 g l⁻¹), held at 10°C for 10 months.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
No human or animal experiments were conducted in this study.

References

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