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Reclassification of Halothiobacillus hydrothermalis and Halothiobacillus halophilus to Guyparkeria gen. nov. in the Thioalkalibacteraceae fam. nov., with emended descriptions of the genus Halothiobacillus and family Halothiobacillaceae

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Abstract:	The genus Halothiobacillus contains 4 species of obligate autotrophs with validly published names, of which Halothiobacillus halophilus and Halothiobacillus hydrothermalis are very distant from the type species - on the basis of the 16S rRNA gene, they have 90.7 % and 90.9 % identity to that of the type species, Halothiobacillus neapolitanus. As these values fall below the Yarza cut-off for the rank of genus, and these two species also show no clear affiliation to the closely related genus Thioalkalibacter, a polyphasic study was undertaken to determine if they represent a separate genus. Unlike Halothiobacillus spp. sensu stricto, H. halophilus and H. hydrothermalis are halophilic (rather than halotolerant) and moderately alkaliphilic (rather than neutrophilic) and additionally do not produce tetrathionate as a detectable intermediate of thiosulfate metabolism, indicating some significant metabolic differences. On the basis of these data and of functional gene examination, it is proposed that they be circumscribed as a new genus Guyparkeria gen.nov, for which the type species is Guyparkeria halophila gen. nov., comb. nov. Additionally, Thioalkalibacter and Guyparkeria gen. nov. fall distant from the Halothiobacillaceae so the Thioalkalibacteraceae fam. nov. is proposed, for which Thioalkalibacter is the type genus. Emended descriptions of Halothiobacillus, Halothiobacillus neapolitanus and the Halothiobacillaceae are provided.

Reclassification of *Halothiobacillus hydrothermalis* and *Halothiobacillus halophilus* to *Guyparkeria* gen. nov. in the *Haloalkalibacteraceae* fam. nov., with emended descriptions of the genus *Halothiobacillus* and family *Halothiobacillaceae*.

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Abstract

The genus *Halothiobacillus* contains 4 species of obligate autotrophs with validly published names, of which *Halothiobacillus halophilus* and *Halothiobacillus hydrothermalis* are very distant from the type species – on the basis of the 16S rRNA gene, they have 90.7 % and 90.9 % identity to that of the type species, *Halothiobacillus neapolitanus*. As these values fall below the Yarza cut-off for the rank of genus, and these two species also show no clear affiliation to the closely related genus *Thioalkalibacter*, a polyphasic study was undertaken to determine if they represent a separate genus. Unlike *Halothiobacillus* spp. *sensu stricto*, *H. halophilus* and *H. hydrothermalis* are halophilic (rather than halotolerant) and moderately alkaliphilic (rather than neutrophilic) and additionally do not produce tetrathionate as a detectable intermediate of thiosulfate metabolism, indicating some significant metabolic differences. On the basis of these data and of functional gene examination, it is proposed that they be circumscribed as a new genus *Guyparkeria* gen.nov, for which the type species is *Guyparkeria halophila* gen. nov., comb. nov. Additionally, *Thioalkalibacter* and *Guyparkeria* gen. nov. fall distant from the *Halothiobacillaceae* so the *Thioalkalibacteraceae* fam. nov. is proposed, for which *Thioalkalibacter* is the type genus. Emended descriptions of *Halothiobacillus*, *Halothiobacillus neapolitanus* and the *Halothiobacillaceae* are provided.

The genus *Halothiobacillus* (Kelly and Wood, 2000 emend. Sievert *et al.* 2000[1, 2]) was circumscribed originally by Kelly and Wood in their seminal taxonomic study of apparent *Thiobacillus* spp., on the basis of phylogenetic positions of 3 *Thiobacillus* spp. that fell within the *Gammaproteobacteria*, rather than the *Betaproteobacteria*, per *Thiobacillus sensu stricto*. There are now 4 species of *Halothiobacillus* with validly published names – *Halothiobacillus neapolitanus* (type species [1], basonym *Thiobacillus neapolitanus* [3]), *Halothiobacillus halophilus* ([1], basonym *Thiobacillus halophilus* [4]), *Halothiobacillus hydrothermalis* (Kelly & Wood, 2000, basonym *Thiobacillus hydrothermalis* [5]) and *Halothiobacillus kellyi*[2]. All members of the genus are halophilic or halotolerant obligate chemolithoautotrophs, assimilating carbon dioxide *via* the Calvin-Benson-Bassham cycle at the expense of the oxidation of reduced sulfur species.

H. neapolitanus [3] was probably isolated originally by Nathansohn [6] from seawater in the Bay of Naples (reviewed in [7]), but the original strain was lost, and Parker noted that his X^T strain had very similar properties and so named the species as pertaining to the Bay of Naples in spite of his strain being from Melbourne, Australia [3]. It is worth noting for the sake of avoiding confusion that this strain was originally coded as strain $X44^T$ [8], which was later abbreviated by the same author to "*Thiobacillus* X" [9], under which name Trudinger conducted his pioneering studies into the biochemistry of sulfur oxidation [10]. It was latterly termed ParkerX^T, probably *via* culture collection catalogue listings [11]. It is important to note that in the early 1960s, the ParkerX^T strain of *H. neapolitanus* was coded "c2" for the sake of Hutchinson's numerical taxonomic study of various sulfur-oxidising *Bacteria* [12]. This coding has unfortunately persisted and, at times, superseded the original designation of X^T, to the stage that genome sequences in public databases still cite the strain erroneously as $c2^T$, causing confusion with the unrelated "C" strain (= DSM 581 = NCIMB 11333) of *H. neapolitanus* (variously "*Thiobacillus* sp. C" and "*Thiobacillus thioparus*" in the earlier

studies) isolated by Kelly [13], and the subject of seminal studies into energy conservation in chemolithoautotrophs ([13-18). Since Wood and Kelly's study in 2000 creating the genus *Halothiobacillus*, 16S rRNA (*rrs*) gene sequencing has confirmed the well-characterised OSWA [19], W5 [20] and C [13] strains are indeed *bona fide H. neapolitanus* strains [11], giving an expanded view of the physiology and properties of this species beyond the type strain.

Phylogenetic analyses were conducted in MEGA 7.0.26 [21], with alignments made using the MUSCLE algorithm [22] without use of any of the pre-sets that increase speed but may reduce accuracy. DNA or amino acyl sequences were curated from the GenBank™ and Integrated Microbial Genomes and Microbiome Samples (IMG/MER) databases. Phylogenetic reconstruction methods were selected after interrogation of each aligned dataset using the 'find best DNA/protein models' component of MEGA, which selects for the model/method and rate distributions that give the lowest Bayesian information criterion (BIC) and thus describe the substitution patterns of the data the best. For 16S rRNA (*rrs*) gene analyses, the Tamura-Nei model [23] was used with a gamma distribution of rates across sites. For amino acyl analyses based on the form IA ribulose bisphosphate carboxylase/oxygenase (EC 4.1.1.39) large subunit (CbbL) from the Calvin-Benson-Bassham cycle of carbon dioxide fixation and the sulfate thiol esterase (SoxB) canonically found in the Kelly-Friedrich pathway of sulfur oxidation, the Le and Gascuel model [24] was used with a gamma distribution of rates across sites. For all analyses, the maximum-likelihood method was used for reconstruction of phylogenetic trees, with pairwise deletion of gaps at 95 % cut-off. 5,000 bootstrap replicates were performed and values at nodes are shown where $\geq$ 70 %. For 16S rRNA gene analyses, neighbour-joining and minimum-evolution methods were also employed, using the same parameters. In each tree, a sequence from *Allochromatium vinosum* DSM 180^{T} from the *Chromatiales* of the *Gammaproteobacteria* as the outgroup. Gene and

protein percentage identities were calculated from pairwise distances determined using MEGA 7.0.26, using the same model as in the phylogenetic trees but with complete deletion of gaps.

Table 1 curates the properties of *Halothiobacillus* spp. and the closely related *Thioalkalibacter halophilus* [25]. As can be seen from the 16S rRNA gene identities in Table 1, the degree of relatedness between taxa is very low. Whilst *H. halophilus* and *H. hydrothermalis* are quite closely related (but are distinct taxa [26]), they fall very distant from the type species, with only 90.7 and 90.9 % identity thereto – as I have noted previously [11]. On the basis of the 'Yarza cut-off' for genus and the 'Yarza medians' for higher taxa which I designated based on the work of Yarza *et al.* [27, 28] and employed in recent studies of *Thiobacillus*, *Annwoodia*, *Thiomicrospira etc* as well as the higher taxa of the *Betaproteobacteria* [28, 29], these two species fall below the Yarza cut-off for members of the same genus as *H. neapolitanus* ParkerX^{T} (which would be 94.50 %) and, indeed, below the Yarza median for members of the same family (92.25 %), though they are clearly of the same order as they fall above that Yarza median (89.20 %) . From Figure 1 it can be seen that in all tree reconstructions, the overall topology is virtually identical with all branches stemming from well-supported nodes. It can be seen that *Thioalkalibacter halophilus* effectively bisects *Halothiobacillus* rendering it polyphyletic. These factors present a case for a re-evaluation of *Halothiobacillus* spp., which I present here. It is worth noting that whilst *H. kellyi* also falls distant from *H. neapolitanus* at 92.7 % identity, which would indicate they are members of the same family but not the same genus, *H. kellyi* Milos BIII^{T} is found only in one public culture collection (= DSM 13162^{T}), thus it is not possible to reclassify this strain into a separate genus at this time owing to the requirements of Rule 30(3)b of the *International Code of Nomenclature of Prokaryotes* (hereafter 'the *Code*'), thus in this study, I concentrate on the other two species.

The species *H. halophilus* and *H. hydrothermalis* are similar in size to *H. neapolitanus* but are very phylogenetically distant from it. The identity of *H. halophilus* to *H. hydrothermalis* is 98.7 %, and their G+C fractions are only 3.2 mol% apart (but are >8.2 mol% from the type species), which implies that they belong to the same genus but probably to two separate species [30], supported further by a DNA-DNA hybridisation (DDH) value of 59 % [26] below the 70 % cut-off for members of the same species [31]. Strains of these two taxa differ physiologically from *H. neapolitanus* and *H. kellyi* in not producing tetrathionate as an intermediate of thiosulfate oxidation, being more alkalitolerant and having an obligate requirement for sodium chloride to be able to grow. The production of tetrathionate is canonically a diagnostic hallmark of the Kelly-Trudinger pathway of sulfur oxidation, and is owing to the oxidation of thiosulfate by thiosulfate dehydrogenase (cytochrome *c*-linked (EC 1.8.2.2)), and strains lacking this feature have been considered as distinct taxa in previous studies (*e.g. Annwoodia vs Thiobacillus* [28]) as it represents a major physiological difference, and potentially a different pathway of sulfur oxidation. These two taxa also have much higher terminal pH values during growth on thiosulfate than *H. neapolitanus* or *H. kellyi*, probably owing to the poor tolerance of acidity that can be seen from their pH ranges of growth. They are also distinct from *Thioalkalibacter halophilus* by virtue of their 16S rRNA gene identities being below the Yarza cut-off for members of the same genus (see Table 1), forming white colonies that become coated with elementary sulfur over time, instead of the red, sulfur-free colonies of *Tab. halophilus*. They are also slightly alkalitolerant rather than alkaliphilic per *Thioalkalibacter halophilus*.

Figure 2 shows maximum likelihood trees reconstructed from MUSCLE alignments of amino acyl sequences derived from *cbbL* gene. In addition to the clade of interest, I have included *Thiomicrospira* (*Tms*), *Hydrogenovibrio* (*Hgv*) and *Thiomicrorhabdus* (*Tmr*) sequences from the *Gammaproteobacteria* per a subset of the sequences in the CbbL tree used in our recent

study (Supplementary Figure S1 of [29]) since I am confident of both the topology of their CbbL tree and their lines of descent into genera from that study. Given all of the *Halothiobacillus* and *Thioalkalibacter* CbbL sequences are form IAc (*i.e.* carboxysome-associated) RuBisCO (*cf.* the complete Supplementary Figure S1 in [29]), we can assume that all members of these genera use carboxysomes in the fixation of carbon dioxide. The CbbL sequences from the three *Halothiobacillus* species cluster together on a well-supported branch, with *H. hydrothermalis* and *H. halophilus* having 100.0 % identity between CbbL amino acyl sequences (99.9% gene identity – the only difference being *H. hydrothermalis* having GAC as the second codon of *cbbL*, whereas *H. halophilus* has GAT, both encoding asparagine), but they both had 96.3 % identity to that from *H. neapolitanus*. Interestingly, *Tab. halophilus* only had 71.6 % CbbL identity to that of *H. neapolitanus* and 73.1 % to that of *H. hydrothermalis* and *H. halophilus*, instead clustering in the tree with the genus *Hydrogenovibrio*, with 90.0 % amino acid sequence identity to the CbbL from *Hgv. halophilus*, another halophile – this could potential indicate horizontal transfer or specific CbbL evolutionary adaptations to their common environmental conditions. By interrogation of the section of the CbbL sequences of *H. halophilus* and *H. neapolitanus* that aligned (*i.e.* the same 262 aa region), using the ExPASy ProtParam tool [32], both had near identical fractions of positively and negatively charged amino acids, with the *H. neapolitanus* having 34 negatively charged (aspartate and glutamate) residues *versus* 33 in *H. halophilus*, and both having 26 positively charged (arginine and lysine) residues, equal numbers of cysteines *etc*. The former had a predicted pI for the region examined of 6.00 *versus* 5.89 for the latter – this was the only obvious functional difference.

Figure 3 shows maximum likelihood trees reconstructed from MUSCLE alignments of amino acyl sequences derived from *soxB* gene. In this tree, SoxB from *Tab. halophilus* clusters with those of *Halothiobacillus* species and not with *Hydrogenovibrio* species. *H. neapolitanus* and

H. hydrothermalis sequences cluster separately, with 77.0 % identity between amino acyl sequences; 69.2 % identity between SoxB from *H. neapolitanus* and *H. kellyi*, and 63.6 % between *H. neapolitanus* and *Tab. halophilus*. These are very divergent sequences, and similar to identities between SoxB from *Tms. pelophila* and *Tmr. chilensis* (type species of former and closet relative to type species of latter genus for which SoxB sequence available), *viz*. 71.9 %, or between *Tms. pelophila* and *Hgv. marinus* (type species), *viz*. 76.7 %. These data would indicate that the relationships between SoxB and CbbL amino acid sequences of *H. neapolitanus*, *Tab. halophilus* and the *H. hydrothermalis*/*H. halophilus* clade are of even lower percentage identities than one would find between members of different genera of the same family of the *Gammaproteobacteria*, thus supporting the conclusions drawn from 16S rRNA gene studies.

From these data, it can be seen than in terms of physiology, physical properties and phylogenetics, *H. hydrothermalis* and *H. halophilus* do not belong to either of the genera *Halothiobacillus* or *Thioalkalibacter*, thus I propose that they are circumscribed as a separate genus. As the type species *H. neapolitanus* remains in the other genus, under Rule 39a of the *Code*, that taxon must retain the name *Halothiobacillus*. For the novel genus, I propose it be named for Mr Cecil David 'Guy' Parker (1912-1981), Australian microbiologist who discovered *Halothiobacillus neapolitanus* ParkerX^T. Under Rule 10a of the *Code*, "*Parkeria*", "*Parkera*" *etc* cannot be used owing to already being in use in the *Eukarya*, thus I propose *Guyparkeria* gen. nov. The type species is *Guyparkeria halophila* gen. nov., comb. nov., on the basis of the species with the oldest validly published name.

In terms of higher taxa, the relationships of 16S rRNA gene pairwise distances to the Yarza medians indicate that *Guyparkeria* gen. nov. belongs to the same family as *Thioalkalibacter* but not *Halothiobacillus*, as supported by fundamental different properties as curated in Table 2. Thus, *Halothiobacillus* will remain in the *Halothiobacillaceae* [33], but the other two

genera are circumscribed as the *Thioalkalibacteraceae* fam. nov. According to www.bacterio.net, the genera *Thiofaba* and *Thiovirga* also belong to the *Halothiobacillaeae*, but with very low pairwise 16S rRNA gene identities of 87.70 % and 85.30 % from the type strains of their type species to that of *Halothiobacillus*, implying that they may not belong in the same order (Yarza median 89.20 %) or possibly not the same class (86.35 %). They also fall distant from one another at 87.00 %, again implying that they are not in the same order as one another. The exact positions of these genera within the '*Proteobacteria*' will be the result of further study of higher taxa, but it is clear that they are not part of the *Halothiobacillaceae* or the *Thioalkalibacteraceae* fam. nov., so I consider them to be *incertae sedis* pending further work. I also provide an emended description of *H. neapolitanus* to consolidate new data and properties of the well-characterised C (= DSM 581 = NCIMB 11133 [13]), W5 (= LMD 94.73 [20]) and OSWA (= DSM 16832 = ATCC BAA-1086 [19]) strains.

Description of *Thioalkalibacteraceae* fam. nov.

Thioalkalibacteraceae (Thi.o.al.ka.li.bac.te.ra.ce'ae. N.L. masc. n. *Thioalkalibacter*, type genus; *-aceae* suffix to denote family; N.L. fem. pl. n. *Thioalkalibacteraceae* the *Thioalkalibacter* family).

This family is circumscribed on the basis of 16S rRNA gene sequences and comprises the genera *Thioalkalibacter* (type genus) and *Guyparkeria*. Obligate autotrophs using thiosulfate and other sulfur oxyanions, elementary sulfur and sulfide as electron donors. Fix carbon dioxide *via* the Calvin-Benson-Bassham cycle and use form IAc RuBisCO and thus carboxysomes. Ubiquinone-8 (UQ-8) is the dominant respiratory quinone. G+C fractions are from 54 – 68 mol%.

Type genus: *Thioalkalibacter* Banciu *et al*. 2009

Description of *Guyparkeria* gen. nov.

Guyparkeria (Guy.par.ke'ri.a. N.L. fem. n. *Guyparkeria*, named to honour Mr Cecil David 'Guy' Parker (1912-1981), Australian microbiologist who made significant advances in the understanding of sulfur oxidation, concrete corrosion and the taxonomy of the sulfur *Bacteria*)

Members of the *Gammaproteobacteria*, falling within the family *Thioalkalibacteraceae*. Cells are rod shaped, 0.3 – 0.6 μm by 1.0 – 1.5 μm. They are Gram-stain-negative and occur singly or in pairs or short chains and are rapidly motile by means of polar flagella. Do not form endospores or exospores. All members of the genus are strict aerobes which do not denitrify but some species will reduce nitrate to nitrite under oxic conditions. Some species can use nitrate as a nitrogen source; all species can use ammonium. Growth is obligately chemolithoautotrophic at the expense of the oxidation of thiosulfate, tetrathionate, elementary sulfur, sulfide. During growth on thiosulfate, elementary sulfur is formed in the medium, often floating as a pellicle, but tetrathionate, trithionate or pentathionate are not detectable in the medium. Thiocyanate is not used as an energy source, nor are ammonium or ferrous iron ions, carbon disulfide, dimethylsulfide or dimethyldisulfide. Sulfate is the end product of sulfur oxidation, with concomitant increase in culture acidity, with an end point of pH 4.8 – 6.0. Alkalitolerant, with optimal growth occurring from pH 7.0 – 8.5, but growth still occurs at pH 9.0 in some species. Mesophilic, with optimal growth at 30 – 40 °C, with some species moderately thermotolerant, growing at 49 °C. Obligately halophilic, requiring sodium chloride (NaCl) for growth, with optimal growth at 430 – 1,000 mM and maxima of 2,000 – 4,000 mM. Carbon dioxide is fixed using the Calvin-Benson-Bassham cycle, containing form IAc RuBisCO. On thiosulfate agar, colonies are entire, smooth and < 3 mm in diameter, off-white but becoming coated in white and/or yellow elementary sulfur during growth, but colonies themselves do not change colour with age. pH of agar is lowered during growth,

sufficiently to change bromocresol purple from purple to yellow. Ubiquinone 8 (UQ-8) is the dominant respiratory quinone. The G+C fraction of genomic DNA is 64.2 – 67.4 mol %. The 16S rRNA gene has *c*. 91 % identity to that from *Halothiobacillus neapolitanus*. Can be isolated from salt lakes and deep sea hydrothermal vents.

The type species is *Guyparkeria halophila*

Description of *Guyparkeria halophila* comb. nov.

Guyparkeria halophila (ha.lo'phi.la. Gr. masc. n. *hals*, *halos* salt; N.L. fem. adj. *phila* from Gr. adj. *philos* friend, someone dearly loved; N.L. fem. adj. *halophila* salt-loving).

Basonym: *Halothiobacillus halophilus* Kelly and Wood 2000

Gram-stain-negative. Short rods 0.3-0.5 × 1.0-1.2 µm. Motile by means of a single polar flagellum. Colonies grown on basal salts agar supplemented with thiosulfate are 1-3 mm, circular, convex, opaque and smooth, becoming yellow or white with age owing to the deposition of elementary sulfur. Tetrathionate or other polythionates are not detected in cultures grown on thiosulfate, but elementary sulfur is formed. pH of thiosulfate-grown cultures drops to 5.5-6.0 with the cessation of growth. Obligately chemolithoautotrophic. Elementary sulfur, sulfide, thiosulfate, trithionate, tetrathionate and hexathionate but not thiocyanate are used as electron donors. Molecular oxygen is the only terminal electron acceptor. No growth on sugars, amino acids, intermediates of Krebs' cycle, fatty acids, C_1 compounds or complex media. Type strain reduces nitrate to nitrite. Ammonium is used as nitrogen sources. Growth occurs from 26 – 36 °C (optimum 30 – 32 °C) and up to pH 8.4 (optimum pH 7.0 – 7.3). Obligately halophilic with an optimum of 1.0 M (5.8 % *w/v*) NaCl and a maximum of 4.0 M (23.2 % *w/v*). Endospores, exospores, cysts and capsules are not produced. Dominant respiratory quinone is ubiquinone-8 (UQ-8).

G+C fraction of genomic DNA of the type strain is 64.2 mol% (HPLC).

The type strain is 204^T = DSM 6132^T = ATCC 49870^T (isolated from the waters of Lake O'Grady, a hypersaline (*c.* 6 % *w/v* NaCl [34]) playa in the Shire of Koorda in the Wheatbelt of Western Australia, Australia).

Description of *Guyparkeria hydrothermalis* comb. nov.

Guyparkeria hydrothermalis (hy.dro.ther.ma'lis. N.L. fem. adj. *hydrothermalis* hydrothermal, pertaining to a hydrothermal vent).

Basonym: *Halothiobacillus hydrothermalis* (Durand *et al.*, 1997; Kelly and Wood, 2000)

Gram-stain-negative. Short rods 0.5 × 1.0 µm. Motile by means of a single polar flagellum. Colonies grown on basal salts agar supplemented with thiosulfate are 1-3 mm, circular, convex, opaque and smooth, becoming yellow or which with age owing to the deposition of elementary sulfur. Tetrathionate or other polythionates are not detected in cultures grown on thiosulfate, but elementary sulfur is formed. pH of thiosulfate-grown cultures drops to 4.8 with the cessation of growth. Obligately chemolithoautotrophic. Elementary sulfur, sulfide, thiosulfate and tetrathionate but not thiocyanate are used as electron donors. Molecular oxygen is the only terminal electron acceptor. No growth on sugars, amino acids, intermediates of Krebs' cycle, fatty acids, C_1 compounds or complex media. Type strain reduces nitrate to nitrite. Ammonium is used as nitrogen sources. Growth occurs from 11 – 45 °C (optimum 35 – 40 °C) and 6.0 – 9.0 (optimum pH 7.5 – 8.0). Obligately halophilic with an optimum of 0.43 M (2.5 % *w/v*) NaCl and a maximum of 2.0 M (11.6 % *w/v*). Endospores, exospores, cysts and capsules are not produced. Dominant respiratory quinone is ubiquinone-8 (UQ-8).

G+C fraction of genomic DNA of the type strain is 67.4 mol% (HPLC).

The type strain is R3^T = DSM 7121^T = ATCC 51453^T, isolated from samples of hydrothermal vent chimneys taken from an active vent in a rift system of the North Fiji Basin, Pacific Ocean.

Emended description of *Halothiobacillaceae* Kelly and Wood 2005

Halothiobacillaceae (Ha.lo.thi.o.ba.cil.la.ce'ae. N.L. masc. n. *Halothiobacillus*, type genus; -*aceae* suffix to denote family; N.L. fem. pl. n. *Halothiobacillaceae* the *Halothiobacillus* family).

This family is circumscribed on the basis of 16S rRNA gene sequences and comprises the genus *Halothiobacillus* (type genus). Obligate autotrophs using thiosulfate and other sulfur oxyanions, elementary sulfur and sulfide as electron donors. Obligate aerobes using only molecular oxygen as a terminal electron acceptor. Fix carbon dioxide *via* the Calvin-Benson-Bassham cycle using form IAc RuBisCO and thus carboxysomes. Ubiquinone-8 (UQ-8) is the dominant respiratory quinone. G+C fractions of genomic DNA are typically from 54 – 62 mol%.

Type genus: *Halothiobacillus* Kelly and Wood 2000

Emended description of *Halothiobacillus* Kelly and Wood 2000 emend. Sievert *et al.* 2000

Halothiobacillus (ha.lo.thi.o.ba.cil'lus. Gr. masc. n. *hals*, *halos* salt; Gr. neut. n. *theion* brimstone, sulfur (Latin transliteration *thium*), L. masc. n. *bacillus* a small rod; N.L. masc. n. *Halothiobacillus* salt-loving sulfur rodlet).

Members of the *Gammaproteobacteria*, falling within the family *Halothiobacillaceae*. Cells are rod shaped, 0.3 – 0.5 μm by 1.0 – 1.5 μm. They are Gram-stain-negative and occur singly or in pairs or short chains and are motile by means of a single polar flagellum. Do not form endospores, exospores, capsules or cysts. All members of the genus are strict aerobes which do not denitrify. Can use ammonium, nitrate or nitrite as sole nitrogen sources. Growth is obligately chemolithoautotrophic at the expense of the oxidation of thiosulfate, tetrathionate, elementary sulfur, sulfide. During growth on thiosulfate, elementary sulfur is formed in the medium, often floating as a pellicle, and tetrathionate is detectable in the medium in the first 24h in aerated cultures, but is then further oxidised, but does remain detectable in static cultures. Produces carboxysomes, formation of which can be repressed by growth at elevated carbon dioxide partial pressures. Thiocyanate is not used as an energy source, nor are ammonium ferrous iron, carbon disulfide, dimethylsulfide or dimethyldisulfide. Sulfate is the end product of sulfur oxidation, with concomitant increase in culture acidity, with an end point of pH 2.8 – 3.0. Acidotolerant, with optimal growth occuring at pH 6.5 – 6.9, but growth still occurs from pH 4.5 – 8.5. Mesophilic, with optimal growth at 28 – 32 °C, with growth still occurring at 39 °C. Moderately halotolerant, not requiring sodium chloride (NaCl) for growth and tolerating it up to 840 mM. Carbon dioxide is fixed using the Calvin-Benson-Bassham cycle. On thiosulfate agar, colonies are entire, smooth, glistening and < 4 mm in diameter, off-white but becoming coated in white and/or yellow elementary sulfur during growth, and colonies turn pink in the centre with age. pH of agar is lowered during growth, sufficiently to change bromocresol purple from purple to yellow. Ubiquinone 8 (UQ-8) is the dominant respiratory quinone. The G+C fraction of genomic DNA is around 56 mol%. Can be isolated from decomposing concrete, seawater, soils and freshwater.

Type species: *Halothiobacillus neapolitanus* Kelly and Wood 2000

Emended description of *Halothiobacillus neapolitanus*

Halothiobacillus neapolitanus (ne.a.po.li.ta'nus, L. masc. adj. *neapolitanus*, of or pertaining to *Neapolis* (Naples, city in *Regio Latium et Campania*, Roman *Italia*), Neapolitan, in this case referring to the seawater of the Bay of Naples from which Alexander Nathansohn probably isolated this species in 1902).

Gram-stain-negative. Short rods 0.3-0.5 × 1.0-1.5 µm. Type strain is very rapidly motile reaching speeds of up to 0.15 mm/s, such that individual cells can be hard to see clearly in wet-mounts unless poisoned with cyanide or azide, but other non-motile strains have been described. Colonies grown on basal salts agar supplemented with thiosulfate are 1-2mm, circular, convex and glistening, white-to-off-white and yellowing with age owing to the deposition of elementary sulfur. Young colonies may have orange centres to transmitted light, and older colonies become pink in the centre with age. In static cultures in basal salts liquid media supplemented with thiosulfate, elementary sulfur, trithionate and tetrathionate commonly accumulate, and a uniform pellicle of elementary sulfur is formed. Well-aerated cultures will show a transient accumulation of trithionate and tetrathionate. Continuous-flow chemostat cultures using thiosulfate as the sole electron donor do not accumulate any detectable intermediates and thiosulfate is stoichiometrically converted to sulfate. pH of thiosulfate-grown cultures drops to 2.8-3.3 with the cessation of growth. Packed cells harvested from thiosulfate cultures are orange with absorbance maxima of whole cells at 522 and 551 nm, corresponding to the β and α bands of cytochrome *c*, respectively. Obligately chemolithoautotrophic but cells grown in the presence of thiosulfate can assimilate carbon from acetate but not glucose. Elementary sulfur, sulfide, thiosulfate, trithionate and tetrathionate but not thiocyanate, dithionate or sulfite are used as electron donors. Weak growth on thioacetamide. Rapid production of elementary sulfur from sulfide is seen in some strains. Molecular oxygen is the only terminal electron acceptor. Has *bd*-I type ubiquinol

oxidase and *cbb*3-type cytochrome *c* oxidase genes, with activity of the latter shown *in vivo* in at least one strain. No growth on sugars, amino acids, intermediates of Krebs' cycle, fatty acids, C_1 compounds or complex media. Type strain does not reduce nitrate to nitrite but slight reduction is observed in other strains. Fixes carbon dioxide using the Calvin-Benson-Bassham cycle (transaldolase variant) and forms carboxysomes ('polyhedral bodies'). Has form IAc RuBisCO. Ammonium, nitrate and nitrite are used as nitrogen sources, with ammonium giving greater yields. Growth occurs from 8 – 39 °C (optimum 28 – 32 °C), from pH 4.5 – 8.5 (optimum pH 4.5 – 8.5). Does not tolerate even brief incubation at 55 °C – death occurs. Halotolerant to 0.86 M (5 % *w/v*) NaCl and solute-tolerant *e.g.* to 0.38 M (6 % *w/v*) Na thiosulfate. Salt not required for growth. Endospores, exospores, cysts, capsules and volutin (polyphosphate) granules are not produced. Dominant respiratory quinone is ubiquinone-8 (UQ-8). Readily isolated from marine mud; canal, pond and river waters; seawater; soils; sulfidic wells/springs.

G+C fraction of genomic DNA of the type strain is 54.7 mol% (from the genome sequence).

Type strain: X^T = ParkerX^T = $c2^T$ = CIP 104769^T = DSM 15147^T = NCIMB 8539^T, isolated from decomposing concrete in the outfall sewer of south east Melbourne, Australia.

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Conflicts of Interest

The author declares that he has no conflict of interest.

Ethical Statement

No experiments with humans or animals were carried out.

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Table 1. Comparitive properties of *Halothiobacillus* and *Thioalkalibacter* species. Data refer to type strains unless otherwise indicated.

Data are curated from Banciu [25], Boden *et al*., [11], Kelly [13], Durand *et al.* [5], Parker [3], Sievert *et al.* [2], Wood and Kelly [4], Wood and Kelly [1], Wood *et al.*, [19].

UQ-8, ubiquinone-8; *N.D.*, not determined/no data available; +, positive or present; -, negative or absent; ±, weakly positive.

* From washout kinetics of thiosulfate-limited chemostat culture rather than batch culture.

† For *H. neapolitanus* OSWA [19] but not the type strain.

‡ For *H. neapolitanus* strain C [13].

Table 2. Curated properties of the families *Halothiobacillaceae* and *Thioalkalibacteraceae* fam. nov. Data are from Kelly and Wood [33] and the references given for Table 1. Unless otherwise stated, properties relate to batch cultures on thiosulfate as the electron donor, oxygen as the terminal electron acceptor and carbon dioxide as the carbon source.

Figure 1. Phylogenetic trees on the basis of the 16S rRNA (*rrs*) gene, showing the positions of *Halothiobacillus halophilus* DSM 6162^T and *Halothiobacillus hydrothermalis* DSM 7121^T as distinct from *Halothiobacillus* species *sensu stricto* and from *Thioalkalibacter halophilus* ALCO1^T. The type species of *Halothiobacillus* is shown in bold text. Nucleotide sequences were aligned using MUSCLE and trees were reconstructed using the Tamura-Nei model with a gamma distribution across sites, in MEGA 7.0.26, with 5,000 bootstrap replications. Values next to nodes indicate the percentage of reconstructions in which the topology was preserved (values <70 % are omitted for clarity). All positions with <95 % site coverage were omitted from the final analyses, which used 1,351 nt. Branch lengths are to scale and indicate the number of substitutions per site – bars represent 20 substitutions per site on all trees shown. The outgroup of each tree is the 16S rRNA gene from *Allochromatium vinosum* DSM 180^T from the *Gammaproteobacteria*. Maximum likelihood tree shown had highest log-likelihood after 5,000 replications (-4721.41). Neighbour joining and minimum evolution trees shown had the optimal sum of branch length (0.386).

Figure 2. Phylogenetic trees on the basis of amino acyl sequences derived from the type IA ribulose bisphospate carboxylase/oxygenase (EC 4.1.1.39) large subunit gene (*cbbL*) and the sulfate thiol esterase gene (*soxB*), showing *Halothiobacillus* spp. and other halophilic sulfur-oxidising *Gammaproteobacteria*, with the type species of *Halothiobacillus* in bold text. Amino acid sequences were aligned using MUSCLE and trees were reconstructed using the maximum likelihood method and the Le and Gascuel model with a gamma distribution across sites, in MEGA 7.0.26, with 5,000 bootstrap replications. Values next to nodes indicate the percentage of reconstructions in which the topology was preserved (values <70 % are omitted for clarity). All positions with <95 % site coverage were omitted from the final analyses, which used 168 positions for CbbL and 197 for SoxB. Branch lengths are to scale and indicate the number of substitutions per site – bars representing 5 (CbbL) or 10 (SoxB) substitutions per site. Outgroups of each tree are the respective derived amino acyl sequence from the equivalent gene of *Allochromatium vinosum* DSM 180^T from the *Gammaproteobacteria*. The trees shown had the highest log-likelihoods after 5,000 replications, namely -1,297.94 (CbbL) or -3,018.00 (SoxB).

	Halothiobacillus		*Guyparkeria* gen. nov.		*Thioalkalibacter*
Species	***H. neapolitanus***	***H. kellyi***	***H. halophilus***	***H. hydrothermalis***	***Tab. halophilus***
Origin of type strain	Concrete in early stages of corrosion from sewers of Melbourne, Victoria, Australia	Sediment from shallow-water hydrothermal vent, Bay of Palaeochori, Milos, Greece	Water from hypersaline playa Lake O'Grady, Western Australia, Australia	Fragments from chimney of deep-water hydrothermal vent, North Fiji Basin, Pacific Ocean	Pooled sediments from various hypersaline lakes, Altai, Russia
Colonial properties (on basis of growth on thiosulfate as sole electron donor):					
Colour (reflected light)	White, pink centres with age.	White	Off-white, yellowing with age	Off-white	Red
Shape	Circular	Circular	Circular	Circular	*N.D.*
Margin	Entire	Entire	Entire	Entire	*N.D.*
Elevation	Convex	Convex	Convex	Convex	*N.D.*
Lustre/texture	Glistening, but duller/powdery with age	Smooth, but duller/powdery with age	Smooth, but duller/powdery with age	Smooth	*N.D.*
Elementary sulfur	+	+	+	+	-
Dominant respiratory quinones	UQ-8	UQ-8	UQ-8	UQ-8	*N.D.*
Reduction of nitrate to:	-	*N.D.*	Nitrite	-	*N.D.*
16S rRNA (*rrs*) gene identity (%) to that of:					
H. neapolitanus DSM 581^T	100.0	92.7	90.7	90.9	92.7
H. kellyi DSM 13152^T	93.1	100.0	92.6	93.0	91.6
H. halophilus DSM 6132^T	91.3	92.6	100.0	98.7	94.2
H. hydrothermalis DSM 7121^T	91.5	93.0	98.7	100.0	94.3
Tab. halophilus DSM 19224^T	92.2	91.6	94.2	94.3	100.0
Cell properties:					
Diameter (µm)	0.3 – 0.5	0.4 – 0.6	0.3-0.5	0.4-0.6	0.8 – 1.0
Length (µm)	1.0 – 1.5	1.2 – 2.5	1.0-1.2	1.2-1.5	1.5 – 3.0
Cells form short chains	+†	-	+	-	-
Carboxysomes (polyhedral bodies)	+	*N.D.*	*N.D.*	*N.D.*	*N.D.*
G+C fraction (mol%) from lab studies or [genome sequence data]	56.0 [54.7]	62.0	64.2	67.4	54.6
Batch culture on thiosulfate as sole electron donor:					
Tetrathionate detectable	+	+	-	-	-
Elementary sulfur detectable	+	+	+	+	Transient, extracellular.
Inhibition by phenylalanine	-‡	*N.D.*	*N.D.*	±	*N.D.*
pH at end of growth	2.8 – 3.3	2.8 – 3.0	5.5 – 6.0	4.8	*N.D.*
Max. specific growth rate (μ_{max}, h^{-1})	0.280	0.450	0.072*	0.613	0.055
Temp. range (°C):	8-39	37-42	26-36	11-45	*N.D.*
Temp. opt. (°C):	28-32	48-49	30-32	35-40	30
pH range:	3.00 – 8.50	3.50 – 8.50	*N.D.* – 8.40	6.00-9.00	7.50 – 10.05
pH opt.:	6.50 – 6.90	6.50	7.00 – 7.30	7.50-8.50	8.00 – 9.00
Relationship with pH	Neutrophilic	Neutrophilic	Moderately alkalitolerant	Moderately alkalitolerant	Alkaliphilic
NaCl max. (mM):	*N.D.*	2,500	4,000	2,500	3,800
NaCl opt.(mM)	0-860	400-500	1,000	430	1,500
Relationship with NaCl	Halotolerant	Halotolerant	Halophilic	Halophilic	Halophilic

Electron donors (all use thiosulfate but not thiocyanate):					
Trithionate	+	*N.D.*	+	*N.D.*	*N.D.*
Tetrathionate	+	+	+	+	*N.D.*
Hexathionate	*N.D.*	*N.D.*	+	*N.D.*	*N.D.*
Sulfide	+	+	+	+	+
Elementary sulfur	+	+	+	+	+
Nitrogen sources (all use ammonium):					
Nitrate	+	*N.D.*	*N.D.*	+	*N.D.*
Nitrite	+	*N.D.*	*N.D.*	-	*N.D.*

Table 2

	Halothiobacillaceae	***Thioalkalibacteraceae***
Genera	*Halothiobacillus*	*Thioalkalibacter* *Guyparkeria*
Colony colour	White	White, red
Cell diameter × length (µm)	0.3 – 0.6 × 1.0 – 2.5	0.3 – 1.0 × 1.0 – 3.0
Soluble intermediates of thiosulfate oxidation	Trithionate Tetrathionate	-
G+C fraction (mol%)	56.0 – 62.0	54.6 – 67.4
Optimal NaCl concentration (mM)	400 – 860	430 – 1,500
Salt profile	Halotolerant	Obligately halophilic
pH profile	Acidotolerant Neutrophilic	Alkalitolerant Alkaliphilic
Max. specific growth rate on thiosulfate (μ_{max}, h^{-1})	0.28 – 0.45	0.06 – 0.61
pH at end of growth on thiosulfate	2.8 – 3.3	4.8 – 6.0

Figure 1

Click here to download Figure Fig 1.pdf

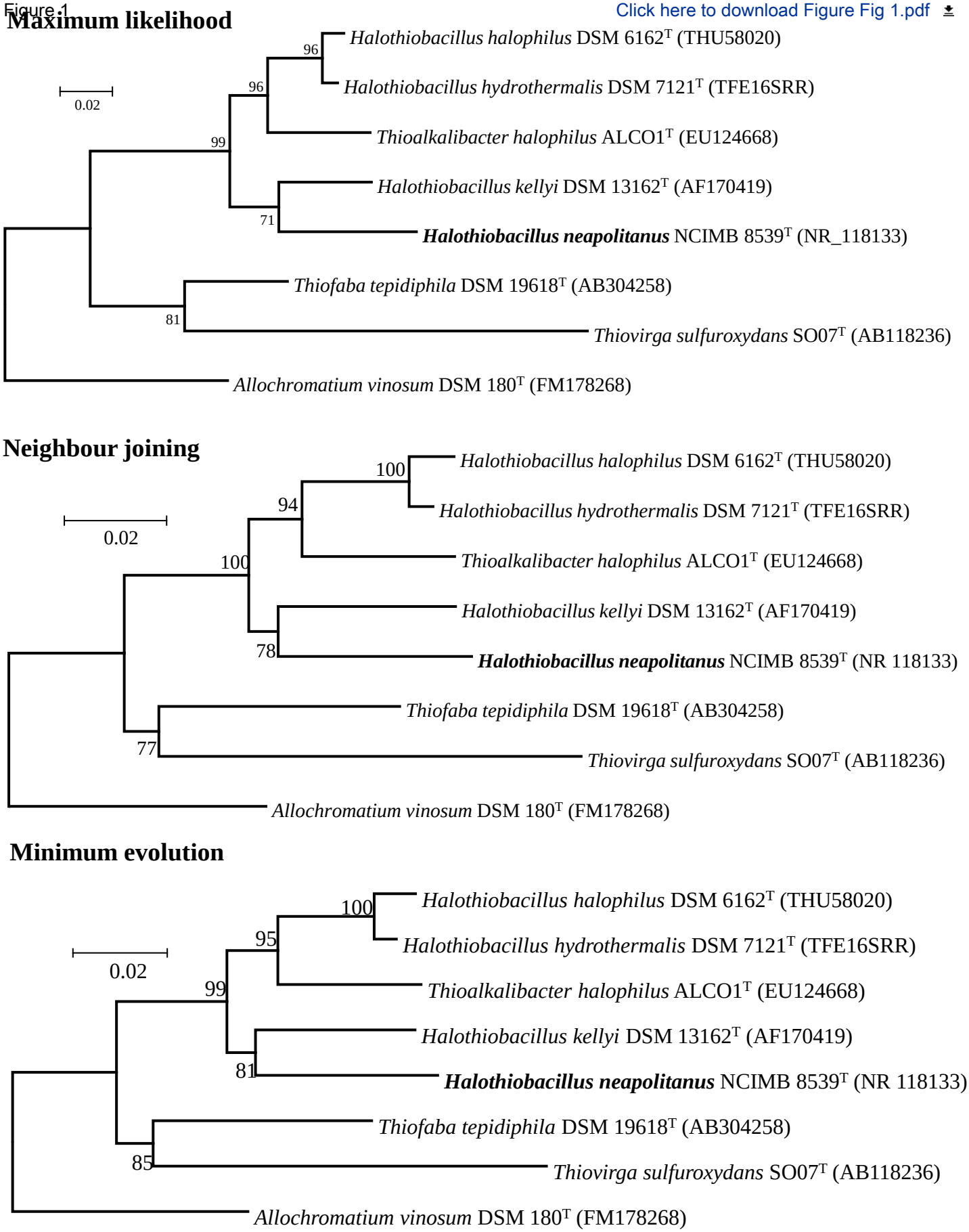
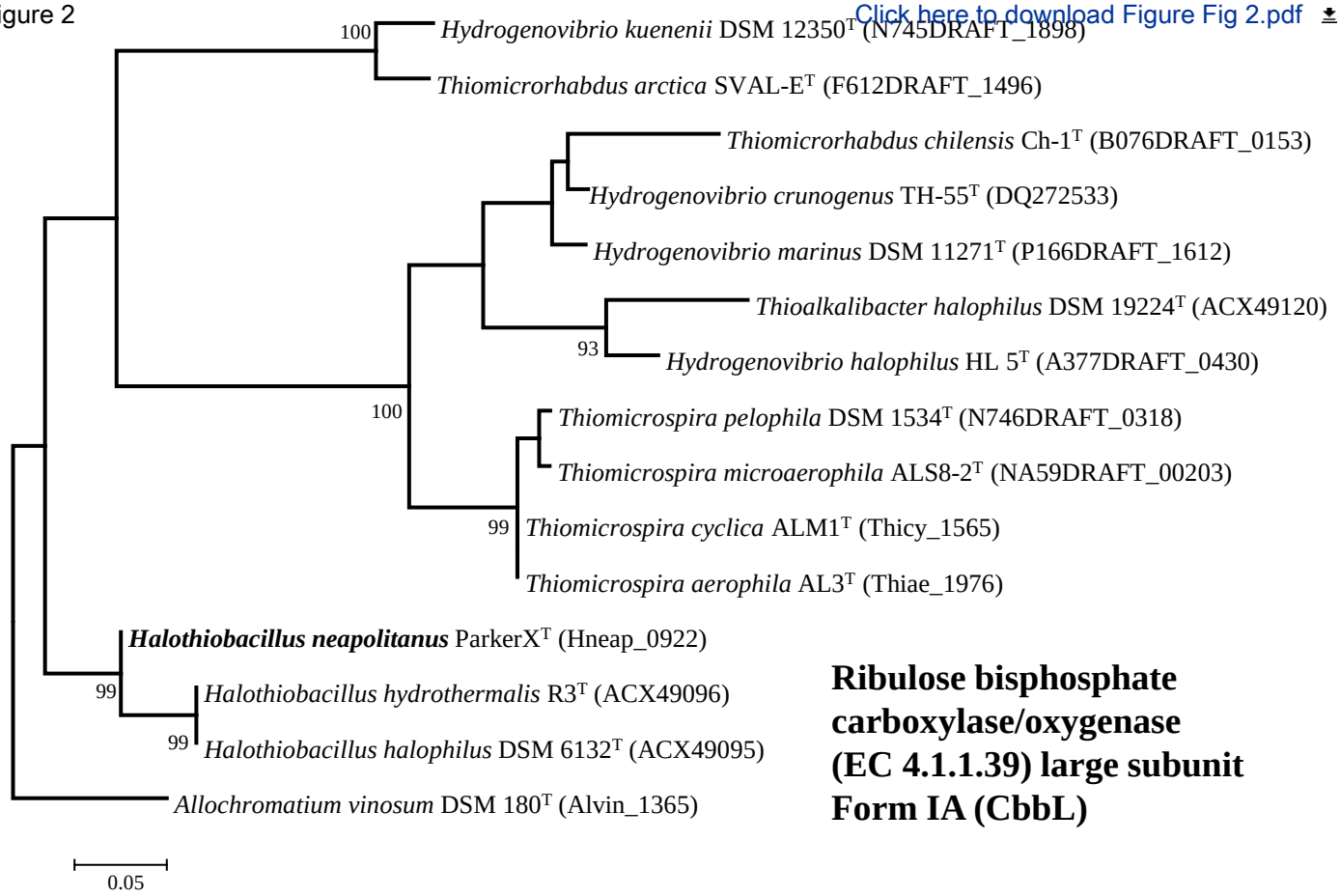
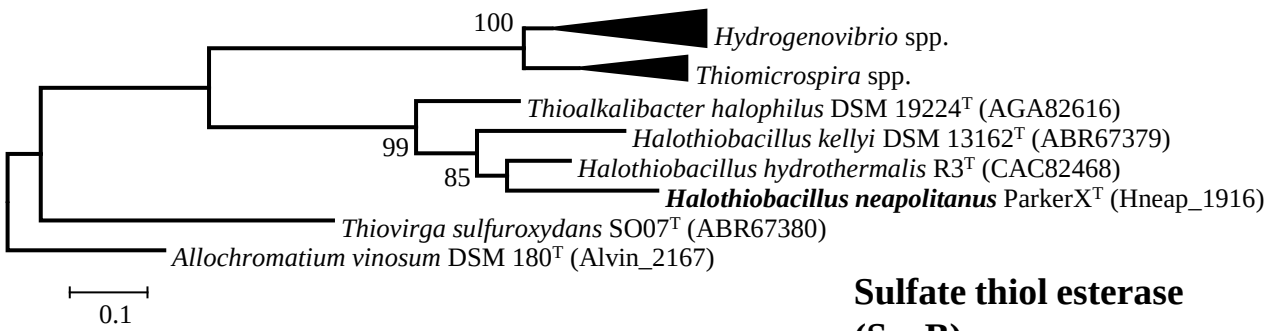


Figure 2



Ribulose bisphosphate carboxylase/oxygenase (EC 4.1.1.39) large subunit Form IA (CbbL)



Sulfate thiol esterase (SoxB)