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# Thiobacillus

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1	Gbm00969
2	Genus Thiobacillus
3	
4	<b>Defining publication:</b> Beijerinck 1904 <i>a</i> , 597 <sup>AL</sup> emend. Boden, Hutt and Rae 2017, 1202.
5	
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11	
12	Etymology:
13	Thi.o.ba.cil'lus. Gr. neut. n. theîon, sulfur, brimstone; L. masc. n. bacillus, a short rod, a short
14	wand; N.L. masc. n. Thiobacillus, sulfur rodlet.
15	Abstract:
16	Cells are short rods. Cytochrome $c$ oxidase-positive and catalase-positive when grown on
17	thiosulfate. Gram-stain-negative. Endospores, exospores and cysts are not produced.
18	Metabolically obligate chemolithoautotrophs, supported by reduced sulfur species and
19	elementary sulfur, and some methylated sulfur compounds. Genes encoding Form IAc, Form
20	IAq and Form II D-ribulose 1,5-bisphosphate carboxylase/oxygenases (RuBisCO) are present in

21 the genomes. Carboxysomes are produced in some species and are repressed at high CO<sub>2</sub> partial

22	pressures. Volutin (polyphosphate) granules formed in most species. Produce tetrathionate as a
23	detectable intermediate of thiosulfate oxidation. Obligately respiratory, with molecular oxygen
24	and nitrate the only known terminal electron acceptors, with the latter only used in some species.
25	Mesophilic, growing optimally at 25-32 °C, and one psychrophilic species capable of growth
26	down to -2 °C. The major respiratory quinone is ubiquinone-8 (UQ-8). Dominant fatty acids are
27	$C_{16:0}$ , $C_{16:1}$ , $C_{15:0}$ and $C_{17:1}$ . The G+C fraction of genomic DNA is 61.5-66.0 mol%.
28	Keywords: aerobe, sulfur, chemolithoautotrophs, organosulfur, denitrifier
29	
30	Description:
31	Cells are short rods of $0.5-0.8 \times 1.0-3.0 \ \mu m$ . Gram-stain-negative. Endospores,
32	exospores and cysts are not produced. Colonies on thiosulfate agar grown at 30 °C are white
33	or colourless and 1-2 mm diameter, circular, convex, smooth and entire, and become covered in
34	powdery white or yellow elementary sulfur over time. Obligately chemolithoautotrophic.
35	Electron donors for chemolithoautotrophic growth include thiosulfate and tetrathionate.
36	Other polythionates, elementary sulfur, as well as some volatile inorganics such as carbon
37	disulfide and carbonyl sulfide are used in some species, and organosulfur compounds such
38	as dimethylsulfide and dimethyldisulfide are used in some strains of Thiobacillus thioparus.
39	Does not grow heterotrophically. Can grow anaerobically with denitrification in some
40	species. Grows optimally at 25-30 °C but growth range varies by species, and from around pH 5
41	to around pH 9, varying by species, with the optima varying by species. Assimilates CO <sub>2</sub> via the

42 transaldolase-variant Calvin-Benson-Bassham cycle. Form II (cytosolic) D-ribulose 1,5-

43	bisphosphate carboxylase/oxygenases	(RuBisCO) is found in all species. Form IAc
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44 (carboxysomal) is found in *T. thioparus* and Form IAq (cytosolic) in all other species.

45

46 *Type species:* **Thiobacillus thioparus** Beijerinck 1904*b*, 153<sup>AL</sup>

47 Number of species with validly published names: 3.

48

### 49 **Family classification**:

50 Thiobacillaceae (fbm00351)

51

## 52 Further Descriptive Information

53 **Morphology**. Cells of *Thiobacillus* spp. are short rods  $(0.5-0.8 \times 1.0-3.0 \,\mu\text{m})$  and are Gram-54 stain-negative. Figure 1 shows transmission electron micrographs of *Thiobacillus thioparus* 55 DSM 505<sup>T</sup> cells obtained from a thiosulfate-limited chemostat. As can be seen from these 56 images, the outer membrane has a 'ruffled' appearance and the periplasm is somewhat dilated as 57 a result – this is visible in both sections shown. The cytoplasm contains numerous carboxysomes ('polyhedral bodies' in the older literature), which usually number c.5 per cell when grown under 58 59 air on thiosulfate. Colonies on thiosulfate agar are circular, entire and rather flat. By reflected 60 light, they are yellowish or pinkish in early growth and then becoming covered in a dense 61 powdery layer of white or yellow elementary sulfur – the colony underneath turns brown with 62 age. By transmitted light, colonies usually look orange or pink, and are darker in color towards 63 the centre. In unshaken batch cultures on thiosulfate, a dense pellicle of cells and elementary

sulfur particles forms – in shaken cultures this is evidenced only as a ring of the same matter on
the glass above the level of the culture.

66 **Metabolism**. As with most known obligate chemolithoautotrophs, Krebs' cycle is incomplete, 67 with Smith's biosynthetic horseshoe (which lacks 2-oxoglutarate dehydrogenase) taking its place 68 (Hutt et al., 2017). Sulfur compound oxidation occurs by way of the Kelly-Trudinger or so-69 called "S<sub>4</sub>!" pathway in which tetrathionate is produced as a detectable intermediate of 70 thiosulfate catabolism and builds up in the medium during the first 24-48 h of batch culture, 71 before being oxidized to sulfate. Degradation of thiocyanate is catalyzed by thiocyanate 72 hydrolase (EC 3.5.5.8) to carbonyl sulfide and ammonia. The former is hydrolyzed by carbonyl 73 sulfide hydrolase (EC 3.13.1.7), forming sulfide and  $CO_2$  – the former being oxidized to sulfate for energy (Yamanaka *et al.*, 2013; Ogawa *et al.* 2013). Proton motive force ( $\Delta p$ ) is generated 74 75 mostly at the level of the terminal oxidase as electrons from thiosulfate oxidation enter the 76 respiratory chain at the level of cytochrome c, thus the next coupling site is the terminal oxidase. 77 NAD(P)H is generated by reverse electron transport. Carbon dioxide is fixed by the transaldolase 78 variant of the Calvin-Benson-Bassham cycle with minor contribution from pyruvate carboxylase 79 and phosphoenolpyruvate carboxylase (Hutt et al., 2017).

80 Nutrition and growth conditions. All species can use thiosulfate or tetrathionate as electron 81 donors for autotrophic growth, and some can also use trithionate, elementary sulfur (*viz.* 82 orthorhombic cyclooctasulfur,  $\alpha$ -S<sub>8</sub>), thiocyanate and other inorganic sulfur species – as shown 83 in Table 1. Use of volatile inorganics carbon disulfide and carbonyl sulfide and organosulfur 84 species such as dimethylsulfide and dimethyldisulfide is of interest since they are trace gases in 85 the atmosphere of Earth. It must be noted that use of organosulfur species in this genus is still 86 *bona fide* chemolithoautotrophy – the (di)sulfide moieties are oxidized to sulfate with

87	concomitant building of proton motive force by the respiratory chain, and thus yielding
88	ATP/NAD(P)H. The methyl groups are oxidized to CO <sub>2</sub> , which is then assimilated <i>via</i> the
89	transaldolase-variant Calvin-Benson-Bassham cycle (Smith and Kelly, 1988). Molecular oxygen
90	and/or nitrate can act as terminal electron acceptors, but the latter is not used in all species.
91	Heterotrophic growth is not observed in this genus. For cultivation, E basal salts (EBS, Boden
92	and Hutt, 2018a) supplemented with 20 mM sodium thiosulfate is commonly used. Other
93	electron donors can be used, usually at 10-20 mM. Elementary sulfur is used at about 0.5 % $w/v$
94	and toxic volatiles at 1-5 mM, but these are 're-spiked' at intervals. Growth is performed under a
95	headspace of air – for the generation of biomass for protein or RNA work, it can be useful to use
96	air enriched with 5 % $v/v$ CO <sub>2</sub> , as this represses carboxysome production in <i>T. thioparus</i> ,
97	simplifying the proteome and transcriptome somewhat. EBS comprises (g/L unless otherwise
98	stated): KH <sub>2</sub> PO <sub>4</sub> (4.0), K <sub>2</sub> HPO <sub>4</sub> (4.0), MgSO <sub>4</sub> ·7H <sub>2</sub> O (0.8), NH <sub>4</sub> Cl (0.4), and trace metal solution
99	T (10 mL). For chemostat work, the addition of a vitamin solution such as solution VJK (Boden
100	and Hutt, 2018a) is prudent but none of the characterized strains of <i>Thiobacillus</i> are auxotrophic
101	for vitamins. Solution T is detailed in the chapter on Annwoodia (gbm01601).
102	Chemotaxonomic features. Similar to most other <i>Betaproteobacteria</i> (cbm00042) and indeed

103 other members of the *Thiobacillaceae* (fbm00351), the dominant fatty acids in *Thiobacillus* spp.

104 are palmitic acid ( $C_{16:0}$ ), palmitoleic acid ( $C_{16:1}$ ), pentadecylic acid ( $C_{15:0}$ ) and heptadecenoic acid

105  $(C_{17:1})$  when grown autotrophically on thiosulfate (Boden et al. 2017) – the latter two fatty acids

106 are distinct from the dominant fatty acids found in the genera *Sulfuritortus* (gbm01835) and

107 Annwoodia (gbm01601). The dominant respiratory quinone is ubiquinone-8, per almost all

108 Betaproteobacteria (cbm00042).

Genomic features. The genome sequences of *Thiobacillus thioparus* DSM 505<sup>T</sup> (Hutt *et al.* 109 2017) and *Thiobacillus denitrificans* ATCC 25259<sup>T</sup> (Beller et al. 2006) have been completed and 110 111 reports of their properties published. We (Boden and Hutt) have recently completed the genome sequence of *Thiobacillus thiophilus* DSM 19892<sup>T</sup> (IMG ID: 168939) and anticipate publication 112 113 of a report of its properties in the near future. We herein curate some findings from these 114 genomes. All species have the *cbb*<sub>3</sub>-type cytochrome *c* oxidase, and *T. thioparus* and *T.* 115 *denitrificans* additionally have the *aa*<sub>3</sub>-type, as shown in Table 1. These indicate (on the basis of 116 Badger and Bek (2008)) that growth is optimized for life at low  $O_2$  partial pressures in all 117 species, but all except T. thiophilus have oxidases optimized for higher O<sub>2</sub> partial pressures as 118 well. Form II (CbbM) RuBisCO is found in all species, optimized for growth at medium to high 119  $CO_2$  partial pressures and low  $O_2$  partial pressures. The non-carboxysome-utilizing T. 120 denitrificans and T. thiophilus also contain Form IAq RuBisCO, optimized for growth at medium 121 to low  $CO_2$  partial pressures, when  $O_2$  is present. T. thioparus has carboxysomes (Figure 1) 122 usually numbering c.5 per cell when grown on thiosulfate under air, which contain Form IAc 123 RuBisCO, optimized for growth at low CO<sub>2</sub> partial pressures in the presence of low-to-high O<sub>2</sub> 124 partial pressures. Across the genus, this indicates that all species are adapted to life under 125 variable gas regimes and all have capacity for life at high CO<sub>2</sub> availability, plus medium-to-low 126 CO<sub>2</sub> availability in *T. denitrificans* and *T. thiophilus*, whereas *T. thioparus* can survive at much 127 lower CO<sub>2</sub> availability. [Ni,Fe]-hydrogenases (EC 1.12.99.6) are found in this genus as well as in 128 the other *Thiobacillaceae* (fbm00351), which could indicate potential for growth on molecular 129 hydrogen as an electron donor, although direct evidence is scant to date. Beller et al. (2006) note 130 that molecular hydrogen-dependent reduction of uranium and other metals occurs in T. 131 *denitrificans*, which could indicate that electrons from molecular hydrogen are used for 'detox'

132 reductases rather than for generation of  $\Delta p$ . Thiosulfate-oxidizing multienzyme system 133 (TOMES) genes are present, arranged soxXYZAB, with soxCD apparently not present. As 134 discussed in Hutt et al. (2017), there is some evidence of the reductase operons associated with denitrification in *T. thioparus* DSM 505<sup>T</sup>, even though this strain does not denitrify in our hands 135 136 or those of previous authors – we have tried under both air and under microxia. 137 Ecology. Thiobacillus spp. can be isolated easily from soils, canal water, river water, reservoir 138 water and from sediments. It is of interest that T. thiophilus was isolated from a BTEX-139 contaminated aquifer (Kellermann and Griebler, 2009), and clone libraries containing 16S rRNA 140 genes with close (>98 % identity) match to *Thiobacillus* spp. have been obtained from tar oil 141 contaminated aquifers (Winderl et al. 2008) - whether Thiobacillus spp. have any metabolic 142 interaction with BTEX compounds and other hydrocarbons is not known, but they may simply 143 not be killed by them. It is also interesting that the other known species of the *Thiobacillaceae* 144 (fbm00351) viz. Sulfuritortus calidifontis and Annwoodia aquaesulis were isolated from thermal 145 springs that flow from deep aquifers, much like T. thiophilus, this observation may indicate 146 populations in the deep subsurface and that the sewage (*T. denitrificans*) and soil (*T. thioparus*) 147 isolates represent descendants of a chthonic lineage.

148 Cultivation, Enrichment and Isolation Procedures

General cultivation: *Thiobacillus* spp. are grown using thiosulfate, tetrathionate, sulfide or
elementary sulfur as the electron donor in a high-phosphate, well-buffered basal salts (E-basal
salts, EBS, is described under *Nutrition and Growth Conditions*). EBS has an ionic strength of
0.314 M and is one of the higher ionic strength media for sulfur oxidizing *Bacteria* – this is
discussed in more detail in the chapter on *Annwoodia* (gbm01601). The two phosphate salts of
EBS are autoclaved separately, in 20 % of the liquid volume. All other ingredients (plus electron)

155 donor and agar, if needed, see below) dissolved in the remaining 80 % of the liquid. After 156 autoclaving at 15 psi for 15 min and cooling completely, the two solutions are mixed and 157 vitamins are added, if using. Thiosulfate and tetrathionate can be autoclaved, and elementary 158 sulfur must be Tyndallized, but all other electron donors should be sterilized by filtration and 159 added from stock solutions after mixing the cooled components. During growth on thiosulfate, 160 the pH of the medium will drop, with growth usually terminating at about pH 5.2 in EBS, but in 161 more weakly buffered media, it will drop to pH 4.5. Elementary sulfur is thrown down during 162 batch cultivation on thiosulfate under air, or at lower oxygen partial pressures, but it can be 163 prevented by growing the organism in the chemostat (Boden and Hutt, 2018a). The most convenient means of growth is to use 50 mL EBS supplemented with the electron donor in 250-164 165 mL Erlenmeyer flasks stoppered lightly with foam or cotton stoppers. Whilst some sources state 166 that shaking cultures of autotrophs will remove  $CO_2$ , but in our hands, very good growth can be 167 obtained by shaking the flasks really rather violently. Baffled flasks (or, in a pinch, the addition 168 of a handful of glass microscope slides or a surgical steel spring to the flask before autoclaving) 169 will produce better growth. It is important to note that in T. thioparus, carboxysomes are formed 170 and their proteins represent a very large fraction of the proteome – it may thus be prudent to use 171 an elevated CO<sub>2</sub> partial pressure for some work in order to repress this.

172**Maintenance:** EBS can be solidified by the addition of 1-2 % (w/v) Noble agar (Sigma or Difco)173or other high purity agar – we use a high purity Granulated Agar by Mellford as well, which174seems to be equally good for *Thiobacillus* spp. but some *Thiobacillus* strains can be 'fussy' and175grow weakly on agar – we have found LUDOX® colloidal silica (W. R. Grace and Co.) to be a176suitable alternative to agar – it is used to replace some of the water of EBS which is then177dispensed into small glass Petri dishes and autoclaved: it solidifies during this process into firm

178 white plates (for full details, refer to the chapter on *Acidithiobacillus* (gbm01079)). Whilst 'nice' 179 colonies are often formed on thiosulfate agar, some *Thiobacillus* spp. grow in the water of 180 syneresis that forms on the top of the agar over time. This gives growth as a white or yellow 181 'smear' rather than proper colonies. The addition of a pH indicator can make distinguishing of 182 growth on agar plates much easier - bromocresol purple is best in our hands as it changes at pH 183 5.2 from violet to yellow - via grey - and does not inhibit growth - we use 5 mL/L of medium of 184 the 0.04 % w/v pharmaceutical grade stock sold by Sigma-Aldrich as it does not contain 185 appreciable organic contaminants. For short-term storage, agar slants or plates can be stored at 4 186 °C for up to a month or so. For medium-term storage, liquid cultures can be grown on e.g. 20 187 mM thiosulfate and then, at late exponential phase, are brought back to pH 7.2 by the dropwise 188 addition of sterile bicarbonate solution – the cultures are then supplemented with thiosulfate to 189 10 mM and stored in the fridge where they will slowly lower the pH once more but remain viable 190 for 6-8 weeks (sometimes longer) before subculture is needed – which will be indicated by the 191 pH indicator turning yellow once more. Longer term, many strains are not lyophilizable or do not 192 freeze well, and thus the service collections provide them as a live culture. We store *Thiobacillus* 193 *thioparus* strains at -80 °C by adding glycerol to cultures to 10 % v/v and resting in ice for 1 h 194 before freezing in liquid nitrogen and slowly 'warming' to -80 °C in dry ice, prior to storing 195 them at this temperature, where they last at least a few years – very high purity 196 dimethylsulfoxide (DMSO) is also a useful cryoprotectant, but some strains do not tolerate it. If 197 in doubt, consultation with the staff at a service collection that holds *Thiobacillus* isolates will 198 usually provide useful 'tricks' – at the time of writing, the staff at the DSMZ in Germany are 199 particularly knowledgeable and helpful in this area.

200 **Enrichment and isolation:** *Thiobacillus* spp. can be enriched on EBS supplemented with a 201 suitable electron donor and incubated under air, or, for the denitrifying species, with the addition 202 of 30 mM potassium nitrate, incubated in completely-filled, sealed bottles of strong glass. 203 Electron donors suitable for isolation include thiosulfate (10-20 mM) or tetrathionate (5-10 mM) 204 but volatile inorganics such as carbon disulfide or carbonyl sulfide or organics such as 205 dimethylsulfide (1-5 mM, monitored and re-spiked 2-3 times between each subculture - cf. 206 Boden and Hutt (2018b) for analytical methodologies) can also be used. Volatile electron donors 207 must be added to cold EBS after autoclaving – it is most convenient to add them "neat" from the 208 bottle (assuming analytical grade to be effectively sterile!) straight into culture flasks - which 209 should be 'QuickFit' Erlenmeyers sealed with 'SubaSeal' vaccine stoppers that have been coated 210 with PTFE Dry Lubricant Spray on the inside before autoclaving. 45 mL volumes of EBS 211 containing the appropriate electron donor are dispensed into Erlenmeyer flasks (see above for 212 special instructions re: volatile substrates) with loose cotton or foam stoppers – we find 'wide 213 mouth' flasks particularly useful for autotrophs, as they promote better  $CO_2$  exchange with the 214 atmosphere. 5 mL of the inoculum is added (pond water, lake water etc or for solid samples, we 215 shake 5-10 g e.g. soil with 20 mL sterile water for 1 h then allow to settle and use 5 mL of the 216 supernate as the inoculum) and the flask incubated with shaking at 25-30 °C. For coupling 217 this ulfate oxidation to nitrate reduction, the same mix is prepared and then poured into c.30-mL 218 thick-walled Universal bottles, right up to the top, and the lid screwed on tightly. If 219 denitrification occurs, bubbles will be observed on the walls – nitrate and nitrite concentrations 220 can be rapidly approximated in a 1:10 dilution of the culture using QuantoFix nitrate/nitrite 221 determination dipsticks – this allows the worker to know if toxic nitrite is building up, which 222 may indicate subculture is needed, if above 10 mM or so. Production of elementary sulfur and/or

223 a pH fall indicate sulfur oxidation is occurring – sometimes the elementary sulfur will disappear. 224 If elementary sulfur (viz. flowers-of-sulfur) is used as the electron donor (at 0.5 g/L), it will float 225 at first but then sink once the organism begins to coat it in surfactants and hydrophilic materials 226 - but turbidity is usually not obvious in these cases. Before subculturing cultures grown on 227 sulfur, vigorous vortex mixing can be used to release cells, or, rather than transferring just 228 supernate, transfer some of the sulfur as well. Serial subculture (10 % v/v at each step) is conducted 5-6 times – by the 4<sup>th</sup> or 5<sup>th</sup>, the time to reach stationary phase is usually about 3-4 229 230 days on thiosulfate/polythionates, a little longer on elementary sulfur, but can be as long as 14 231 days on volatiles as the concentration is lower, they are metabolized more slowly owing to 232 toxicity, and re-spiking is needed. Streak-plates are made on *e.g.* EBS/thiosulfate agar containing 233 bromocresol purple (see above). For denitrifiers, these contain 30 mM potassium nitrate and are 234 incubated under oxygen-free nitrogen or argon (there is no need to use a reducing atmosphere – 235 the molecular hydrogen therein may interfere with some *Thiobacillus* spp. as they contain 236 hydrogenases). For aerobes they are incubated under air, which can be enriched with  $CO_2$  to 5 % 237 v/v, or alternatively, a *small* piece of dry-ice can be put into a box with the plates, or even just a 238 small beaker containing sodium carbonate and a weak mineral acid (Alka-Seltzer tablets and 239 water are commonly used for this purpose, and are quite convenient) – this additional  $CO_2$  often 240 affords bigger colonies and makes the initial isolation step much easier.

For isolates on volatile substrates, EBS agar without an electron donor is used and plates are incubated in metal or plastic gas-jars or in sealed Tupperware<sup>TM</sup> boxes and their atmosphere enriched with the substrate in gas-phase, by using either a substrate "bomb" comprising a glass Bijou tube packed with lens tissue into which 0.5 mL of the neat substrate is added and is then left in the corner of the box, or triangles of sterilized filter paper are put into the inside of the lid

246 of each inverted plate and 1-5  $\mu$ L of the substrate added thereto. This gives excellent growth of 247 Thiobacillus thioparus strains on dimethylsulfide or dimethyldisulfide in our hands. For gases 248 like methanethiol or carbonyl sulfide, a small volume can be added to gas-jars via their ports just 249 after sealing. If plates in boxes or clear gas-jars are incubated either in single layers or interlaced 250 with empty plates, it can be easy to see from the outside which plates have turned yellow 251 (indicating oxidation of sulfur to sulfuric acid), which means one can avoid opening them too 252 frequently and releasing the substrate. Dimethylsulfide and dimethyldisulfide *can* be 253 incorporated into molten agar just before pouring plates, however, we strongly recommend this 254 approach is never taken as the smell permeates the surrounding area, is nauseatingly strong and 255 does not disappear for some days and above all is quite unnecessary – the aforementioned 256 methods give more than satisfactory growth!

### 257 Taxonomic comments

258 Where a three-letter abbreviation is needed to avoid confusion with other genera, we recommend "Tbc." be used. A phylogenetic tree is given in the chapter on Annwoodia (gbm01601), which 259 260 shows the position of *Thiobacillus* spp. and other related taxa. The genus *Thiobacillus* has been 261 changed somewhat over the last 115 years since Beijerinck (1904a, b) first defined it. For most of the 20th century, all sulfur-oxidising Gram-stain-negative organisms were allocated to this 262 263 genus, without regard for most of their properties, though it became clear in the 1980s and 1990s 264 that from the dominant respiratory quinones alone, the organisms in this genus were very 265 diverse. From the early 1990s, studies by Wood and Kelly (1993), Kelly and Wood (2000a,b), 266 Kelly et al. (2000), Katayama et al. (1995), Moreira and Amils (1997) reclassified a large 267 number of Thiobacillus spp. to Paracoccus (gbm00860), Thiomonas (gbm00960), 268 Thiomicrospira (gbm01221), and the newly created genera Acidithiobacillus (gbm01079),

269 Thermithiobacillus (gbm01080), Halothiobacillus (gbm01133) and Starkeya (gbm00828). 270 Further work in the last 5 years by Boden et al. (2017) has reclassified one further species to the 271 newly created genus Annwoodia (gbm01601). As of 2019, the genus Thiobacillus comprises only 272 3 species and we are confident that all 3 are correctly assigned now, with the advent of whole-273 genome sequencing and more advanced phylogenetic methods. It is worth noting that a number 274 of strains in the public service collections that were assigned to *Thiobacillus* were examined by 275 Boden et al. (2012) - in spite of the recent division of the genus with the creation of Annwoodia 276 (gbm01601), most of these *Thiobacillus* strains (viz. E6, Tk-m, Pankhurst T4 and White 2K) are 277 still *Thiobacillus* isolates, and probably affiliate most strongly with *T. thioparus*.

- 278 *List of species in the genus* Thiobacillus
- Thiobacillus denitrificans (ex. Beijerinck 1904b) Kelly and Harrison, 1989, 1855 emend.
   Kelly and Wood 2000a, 548<sup>VP</sup>.
- 281 *de.ni.tri'fi.cans*. N.L. v. *denitrifico*, to denitrify; N.L. part. adj. *denitrificans*, denitrifying.
- 282 Motile rods  $0.5 \times 1.0$ -3.0 µm. Colonies are clear, turning white with age. Optimal growth
- at 28-32 °C and pH 6.8-7.4. Does not produce carboxysomes. Can use thiosulfate,
- tetrathionate, elementary sulfur, thiocyanate, sulfide and pyrrhotite as electron donors for
- chemolithoautotrophic growth, but not dithionate, carbon disulfide, carbonyl sulfide,
- 286 dimethylsulfide, dimethyldisulfide and thiols are not used. Denitrifies, giving vigorous
- evolution of molecular nitrogen when grown on thiosulfate and nitrate in the absence of
- air. Uses Form IAq and Form II RuBisCO and type *aa*<sub>3</sub> and *cbb*<sub>3</sub> cytochrome *c* oxidases,
- does not have a ubiquinol oxidase. Type strain isolated from a sewage lagoon, UK.

290  $DNA \ G+C \ fraction \ (mol\%): 66.07 \ (sequence).$ 

291 *Type strain*: AB7 = ATCC 23644 = CIP 104767 = DSM 12475 = JCM 3870 =
 292 NCIMB 9548.

293 294 GenBank accession (16S rRNA gene): AJ243144

- IMG accession (genome sequence): 637000324
- 295 **2.** *Thiobacillus thioparus* Beijerinck 1904*b*, 153<sup>AL</sup>
- *thi.o'par.us.* neut. n. *theîon*, sulfur, brimstone; N.L. masc. adj. *parus* (from L. v. *paro*, to
  provide, to furnish, to prepare), producing, providing; N.L. masc. adj. *thioparus*, sulfurproducing.
- 299 Motile rods  $0.5 \times 1.7 \,\mu$ m. Colonies are white, turning pink-brown with age. Optimal
- 300 growth at 25-30 °C and pH 6.0-8.0. Produces carboxysomes when grown under air. Can
- 301 use thiosulfate, trithionate, tetrathionate, thiocyanate and sulfide as electron donors for
- 302 chemolithoautotrophic growth. Some strains can use carbon disulfide, carbonyl sulfide,
- 303 dimethylsulfide, dimethyldisulfide and methanethiol, with the former two substrates
- 304 usually giving comparatively weak growth. Dithionate and elementary sulfur are not
- 305 used. Does not denitrify. Uses Form IAc and Form II RuBisCO and type *aa*<sub>3</sub> and *cbb*<sub>3</sub>
- 306 cytochrome *c* oxidases, and *bd*-I ubiquinol oxidase. Type strain isolated from agricultural
- 307 soil, New Jersey, USA.

 308
 DNA G+C fraction (mol%): 62.30 (sequence).

 309
 Type strain: Starkey = ATCC 8158 = CIP 104484 = DSM 505 = JCM 3859 =

 310
 NBRC 103402.

 311
 GenBank accession (16S rRNA gene): HM173629

 312
 IMG accession (genome sequence): 2765235835

- 313 **3.** *Thiobacillus thiophilus* Kellerman and Griebler 2009, 587<sup>VP</sup>.
- 314 *thi.o'phi.lus*. Gr. neut. n. *theîon*, sulfur, brimstone; Gr. masc. adj. *phílos*, dearly loved,
- 315 beloved; N.L. masc. adj. *thiophilus*, sulfur-loving.

316	Motile rods 0.5-0.8 $\times$ 1.8-2.5 $\mu m.$ Colonies are white, turning yellow with age. Optimal
317	growth at 25-30 °C and pH 7.5-8.3. Does not produce carboxysomes. Can use thiosulfate
318	or tetrathionate as electron donors for chemolithoautotrophic growth but does not use
319	elementary sulfur, trithionate, thiocyanate, sulfide, pyrrhotite or molecular hydrogen.
320	Denitrifies, giving vigorous evolution of molecular nitrogen when grown on thiosulfate
321	and nitrate in the absence of air. Uses Form IAq and Form II RuBisCO and type $cbb_3$ and
322	cytochrome $c$ oxidase, and $bd$ -I ubiquinol oxidase. Type strain isolated from a BTEX
323	contaminated aquifer, Germany.
324	DNA $G+C$ fraction (mol%): 62.15 (sequence).
325	<i>Type strain</i> : $D25TN = DSM 19892 = JCM 15047$ .
326	GenBank accession (16S rRNA gene): EU685841
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404	

Character	Thiobacillus thioparus	Thiobacillus denitrificans	Thiobacillus thiophilus
		Jean Jeans	
16S rRNA gene identity of type	100	97.6	97.6
strain to T. thioparus DSM			
505 <sup>1</sup> (%)			
Cell length (µm)	1.7	1.0-3.0	1.8-2.5
Cell diameter (µm)	0.5	0.5	0.5-0.8
Isolation sources of key strains	Agricultural soil, USA;	Sewage lagoon, UK;	BTEX <sup>a</sup> contaminated
in international service	Pond water, UK;	Pond water, Germany;	aquifer, Germany
conections (type strain in bold)	Gasworks sludge, Japan,	Carbonization offluent	
	Soil LIK	lagoon UK:	
	Biofilters (various)	Anaerobic digester UK	
Colony colour (thiosulfate agar.	White, turning	Clear, turning white with	White, turning yellow
under air)	pink/brown with age	age	with age
Use of nitrate as a nitrogen	+	±	N.D.
source			
Use of nitrate as a terminal	-	+	+
electron acceptor			
Temperature range and	<i>N.D.</i>	<i>N.D.</i>	-2-30
[optimum] (°C)	[25-30]	[28-32]	[25-30]
pH range and [optimum]	5.0-9.0	<i>N.D.</i>	6.3-8.7
	[6.0-8.0]	[6.8-7.4]	[7.5-8.3]
Carboxysomes produced	+	-	-
Electron donors for autotrophic g	growth:		
Thiosulfate $(S_2O_3^{2^2})$	+	+	+
Dithionate $(S_2O_6^{-2})$	-	-	N.D.
$\frac{1}{1}$	+	N.D.	-
$\frac{1}{1} = \frac{1}{1} = \frac{1}$	+	+	+
Thiographic (SCN-)	_	+	-
Sulfide (SCN)	+	+	-
Carbon disulfide (CSa)	+	+	
Carbonyl sulfide ( $COS$ )	W	-	N.D.
Methanethiol (CH <sub>2</sub> SH)	w d		N.D.
Dimethylsulfide ((CH <sub>2</sub> ) <sub>2</sub> S)	d d		N.D.
Dimethyldisulfide ((CH <sub>2</sub> ) <sub>2</sub> S <sub>2</sub> )	d d		N.D.
Pyrrhotite (FeS)	N.D.	+	-
Molecular hydrogen (H <sub>2</sub> )	N.D.	N.D.	
D-ribulose 1,5-bisphosphate	Form IAc ( <i>cbbLS</i> )	Form IAa ( <i>cbbLS</i> )	Form IAa ( <i>cbbLS</i> )
carboxylase/oxygenase genes <sup><math>b</math></sup>	Form II ( <i>cbbM</i> )	Form II ( <i>cbbM</i> )	Form II ( <i>cbbM</i> )
Terminal oxidases encoded in genome:			
Cytochrome <i>c</i> oxidases	$aa_3, cbb_3$	$aa_3, cbb_3$	$cbb_3$
Ubiquinol oxidases	bd-I	-	bd-I
G+C fraction (mol%) from	62.30	66.07	62.15
genome			

# **Table 1**. Comparative properties of *Thiobacillus* spp.

*b.* RuBisCO form nomenaclature after Badger and Bek (2008).



414	Figure 1. Transmission electron micrographs of <i>Thiobacillus thioparus</i> Starkey <sup>T</sup> cells
415	obtained from a thiosulfate-limited chemostat, in transverse and longitudinal
416	sections, showing presence of multiple carboxysomes (in the transverse section)
417	and showing the 'puffy' or 'ruffled' periplasm/outer membrane often evident in
418	Thiobacillus spp. Full details of preparation are given in Hutt et al. (2017). Scale
419	bars indicate 200 nm.

420 {Figure 1ab.tif}