Taxonomic Note

Correspondence

Donovan P. Kelly

D.P.Kelly@warwick.ac.uk

Reassessment of the phylogenetic relationships of *Thiomonas cuprina*

Donovan P. Kelly,¹ Yoshihito Uchino,² Harald Huber,³ Ricardo Amils⁴ and Ann P. Wood⁵

¹Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, UK

²NITE Biological Research Center, National Institute of Technology and Evaluation, 2-5-8 Kazasakamatori, Kisarazu-shi, Chiba, 292-0818 Japan

³Lehrstuhl für Mikrobiologie und Archaeenzentrum, Universität Regensburg, Universitätsstraße 31, 93053 Regensburg, Germany

⁴Centro de Biología Molecular (CSIC-UAM), Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain

⁵Department of Microbiology, King's College London, Dental Institute, Floor 28 Guy's Tower, Guy's Campus, London SE1 9RT, UK

The published sequence of the 16S rRNA gene of *Thiomonas cuprina* strain Hø5 (=DSM 5495^T) (GenBank accession no. U67162) was found to be erroneous. The 16S rRNA genes from the type strain held by the DSMZ since 1990 (DSM 5495^T = NBRC 102145^T) and strain Hø5 maintained frozen in the Universität Regensburg for 23 years (=NBRC 102094) were sequenced and found to be identical, but to show no significant similarity to the U67162 sequence. This also casts some doubt on the previously published 5S and 23S rRNA gene sequences (GenBank accession nos U67171 and X75567). The correct 16S rRNA gene sequence showed 99.8 % identity to those from *Thiomonas delicata* NBRC 14566^T and '*Thiomonas arsenivorans*' DSM 16361. The properties of these three species are re-evaluated, and emended descriptions are provided for the genus *Thiomonas* and the species *Thiomonas cuprina*.

The genus *Thiomonas* was proposed by Moreira & Amils (1997) to accommodate four former *Thiobacillus* species. At that time, Moreira & Amils (1997) renamed '*Thiobacillus cuprinus*' as *Thiomonas cuprina* comb. nov., but the name '*Thiobacillus cuprinus*' had never been validly published (Associate Editor, IJSB, 1997), so, while the name '*Thiobacillus cuprinus*' remains the basonym for the species, the new name should be attributed to Moreira and Amils as *Thiomonas cuprina* sp. nov. The four new species names proposed by Moreira & Amils (1997) were *Thiomonas cuprina*, *Thiomonas intermedia*, *Thiomonas perometabolis* and *Thiomonas thermosulfata*. Subsequently, two further species were assigned to the genus: *Thiomonas delicata* and '*Thiomonas arsenivorans*' (Kelly & Wood, 2005; Katayama *et al.*, 2006; Battaglia-Brunet *et al.*, 2006). We have shown that *Thiomonas delicata* and '*Thiomonas arsenivorans*' share >99 % 16S rRNA gene sequence identity (Katayama *et al.*, 2006), indicating a very close phylogenetic relationship. We also previously suggested

that *Thiomonas cuprina* should be considered for reassignment to a new genus, as its published 16S rRNA gene sequence showed only 85–89 % identity to that of any other *Thiomonas* species (Katayama *et al.*, 2006).

We have now carried out a reanalysis of the 16S rRNA gene sequence of *Thiomonas cuprina* strains, and extended the comparison of the 16S rRNA genes of strains of *Thiomonas cuprina*, *Thiomonas delicata* and '*Thiomonas arsenivorans*', in order to clarify the interrelationships of these three species.

First, the 16S rRNA gene sequence (1456 bp) of *Thiomonas cuprina* has been determined for the type strain held by the DSMZ since 1990 (DSM 5495^T = NBRC 102145^T), and for the original strain (Hø5) after maintenance as a frozen stock culture for 23 years in the University of Regensburg (=NBRC 102094). The sequences were identical to each other, but differed significantly from the previously published sequence for *Thiomonas cuprina* DSM 5495^T (GenBank accession no. U67162). Indeed, it transpires that the sequences for strains of *Thiomonas cuprina*, *Thiomonas delicata* and '*Thiomonas arsenivorans*' differ from each other by only three nucleotides in 1455–1456 nucleotides of sequence. By 16S rRNA gene sequences alone, these three species were therefore indistinguishable.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Thiomonas cuprina* strain Hø5 (=DSM 5495^T = NBRC 102145^T) and NBRC 102094 are respectively AB331954 and AB331953.

Consequently, the 16S rRNA gene sequence published by Moreira & Amils (1997) must be discounted: the DNA analysed at that time must have arisen from an organism that was not the type strain of *Thiomonas cuprina*. The U67162 sequence is in fact rather remote from any other proteobacterial sequence on the databases (Y. Uchino, unpublished data), and rediscovery of the organism from which it came would clearly be of interest. Unfortunately, our new finding also means that the published data on the 5S and 23S rRNA gene sequences of the DNA of the organism studied by Moreira *et al.* (1994) and Moreira & Amils (1997), as well as reports on the genomic organization and chromosome size, must be treated with caution and merit further investigation (Moreira *et al.*, 1994; Moreira & Amils, 1996, 1997; Marin *et al.*, 1997). The 23S rRNA gene of *Thiomonas cuprina* DSM 5495^T was sequenced independently by Ludwig *et al.* (1995), using cells provided directly from Regensburg by Karl O. Stetter. This sequence (GenBank accession no. X87292; 2877 nucleotides) shows only 96.9 % identity (2788/2877 aligned bases) to that published by Moreira *et al.* (1994) (GenBank accession no. X75567; 2858 nucleotides). The 90 mismatches include 51 gaps in the sequence alignment. When the X87292 sequence is run in a BLASTN search (Altschul *et al.*, 1997), the nearest matched sequence is, however, X75567, with the next closest hits being to the complete genomes of *Methylobium petroleiphilum* PM1^T (GenBank accession no. CP000555; 91 % to an unnamed region) and *Acidovorax* sp. JS42 (CP000539; 89 % to the 23S rRNA gene). The 5S rRNA gene of their strain was also sequenced by Moreira & Amils (1997) (GenBank accession no. U67161), and shows 91.2 % identity to those of *Thiomonas intermedia* ATCC 15466^T (M11538) and *Thiomonas perometabolis* ATCC 23370^T (M11539), but also 97.4 % identity to that of *Leptothrix discophora* strain Stokes (M35569), so must be viewed with some reservation until it can be re-evaluated.

The species description of *Thiomonas cuprina* requires only minor amendment, as the properties described have been confirmed as those of the strain from which the correct 16S rRNA gene sequences came (Huber & Stetter, 1990). Emendations to the species description include a listing of the current holdings of the type strain, the citation of the accession numbers for its 16S rRNA gene sequence and the omission of the previously published 5S and 23S rRNA gene sequence accession numbers, until these can be re-evaluated.

Given the virtually identical 16S rRNA gene sequences of *Thiomonas cuprina*, *Thiomonas delicata* and '*Thiomonas arsenivorans*', we have summarized the physiological evidence to retain *Thiomonas cuprina* and *Thiomonas delicata* as separate species. Six species of *Thiomonas* have been described (Kelly & Wood, 2005; Battaglia-Brunet *et al.*, 2006; Katayama *et al.*, 2006): some comparative properties are summarized in Table 1. *Thiomonas intermedia* (the type species of the genus) and *Thiomonas perometabolis* share 99.7 % 16S rRNA gene sequence identity (1376/1380 aligned nucleotides) and 100 %

identity (116/116 nucleotides) of their 5S rRNA gene sequences, but are recognized as distinct species, both physiologically and by DNA hybridization (Katayama-Fujimura *et al.*, 1983). *Thiomonas cuprina* shows significant physiological differences from other *Thiomonas* species, including its ability to oxidize and grow on some metal sulfides (including chalcopyrite, with the release of copper, and arsenopyrite) and its inability to oxidize thiosulfate or tetrathionate (Huber & Stetter, 1990). Moreira & Amils (1997) incorrectly described *Thiomonas cuprina* as not growing on sulfide minerals (which it can do), but as being able to grow on pyrite (which it cannot). No other *Thiomonas* species have been reported to grow on chalcopyrite or arsenopyrite, and none can use pyrite as a growth substrate. The supposed ability to grow on pyrite was unfortunately carried over into the chapter on *Thiomonas* in the latest edition of *Bergey's Manual of Systematic Bacteriology* (Kelly & Wood, 2005), and is corrected in the amended species description provided below. The ability to grow on thiosulfate or tetrathionate was reported incorrectly as a genus-wide property in the genus description provided by Moreira & Amils (1997), but was corrected by Kelly & Wood (2005). Ferrous iron [Fe(II)] is also oxidized by some *Thiomonas* species, including *Thiomonas delicata* (K. B. Hallberg, personal communication), but not by *Thiomonas cuprina* (Huber & Stetter, 1990; Kelly & Wood, 2005; Battaglia-Brunet *et al.*, 2006; Katayama *et al.*, 2006). Most strains of *Thiomonas* show optimum growth under mixotrophic conditions, with reduced sulfur compounds and organic supplements (Moreira & Amils, 1997), although one strain has been reported as non-mixotrophic (Pol *et al.*, 2007), and the facultative *Thiomonas cuprina* can grow rapidly on yeast extract as its sole substrate ($\mu=0.17\text{--}0.23\text{ h}^{-1}$; Huber & Stetter, 1990).

There are enough physiological differences between the three closely related species to justify their retention as individual species until further evidence becomes available to assess the relationship between *Thiomonas delicata* and '*Thiomonas arsenivorans*'. It is noteworthy that the arsenic-rich habitats from which '*Thiomonas arsenivorans*' was recovered also harboured other strains showing very high 16S rRNA gene sequence identity to *Thiomonas cuprina*, including strain CO2, with 100 % identity for 1454 aligned nucleotides (GenBank accession no. AF460988; Battaglia-Brunet *et al.*, 2002). Such metal-rich habitats may thus support a diversity of closely related strains with a range of physiological properties, as was noted for the five strains of *Thiomonas cuprina* studied originally (Huber & Stetter, 1990).

An emended description of *Thiomonas cuprina* is given below. The genus description of *Thiomonas* also requires amendment, as the literature review for the description published in *Bergey's Manual of Systematic Bacteriology* (Kelly & Wood, 2005) only included published material available up to September 2000. Since then, the ability of some strains to oxidize Fe(II) and arsenite [As(III)] has been proved (Battaglia-Brunet *et al.*, 2002, 2006; Bruneel

Table 1. Comparative properties of *Thiomonas cuprina* and the five other named species of *Thiomonas*

Strains: 1, *Thiomonas cuprina* DSM 5495^T (=NBRC 102094^T); 2, *Thiomonas delicata* NBRC 14566^T; 3, '*Thiomonas arsenivorans*' DSM 16361; 4, *Thiomonas intermedia* ATCC 15466^T (type species of the genus); 5, *Thiomonas perometabolis* ATCC 23370^T; 6, *Thiomonas thermosulfata* ATCC 51520^T. Data were obtained in the present study and from London (1963), London & Rittenberg (1967), Mizoguchi *et al.* (1976), Huber & Stetter (1990), Katayama-Fujimura & Kuraishi (1983), Katayama-Fujimura *et al.* (1984), Kelly & Harrison (1989), Shooner *et al.* (1996), Kelly & Wood (2005), Kelly *et al.* (2005), Battaglia-Brunet *et al.* (2006) and Katayama *et al.* (2006). All strains show chemolithotrophic growth on/oxidation of elemental sulfur. NA, No data available.

Characteristic	1	2	3	4	5	6
DNA G + C content (mol%)	66	66–67	65	65–67	65–66	61
16S rRNA gene sequence similarity (%) to the sequence of:						
<i>Thiomonas cuprina</i> DSM 5495 ^T (AB331954)	(100)	99.8	99.8	93.6	93.6	90.9
<i>Thiomonas intermedia</i> ATCC 15466 ^T (AY455809)	93.6	93.4	93.5	(100)	99.7	94.1
Cell dimensions (µm)						
Diameter	0.3–0.5	0.4–0.6	0.3–0.5	0.6–0.8	0.4–0.8	0.9
Length	1.0–4.0	1.0–1.6	1.0–2.0	1.0–1.4	1.1–1.7	1.0–4.0
Motility	+	–	+	+	+	+
Flagellum	Polar	–	+	Polar	Polar	+
Ubiquinone	Q-8	Q-8	NA	Q-8	Q-8	Q-8
Chemolithotrophic growth on/oxidation of:						
Thiosulfate	–	+	+	+	+	+
Tetrathionate	–	+	+	+	+	+
Fe(II)	–	+	+	–	–	–
Chemo-organotrophic growth with single organic compounds	+*	–	+	–	+	+†
Nitrate reduction to nitrite	–	+	–	–	–	–
pH for growth						
Optimum	3.0–4.0	5.5–6.0	4.0–7.5	5.5–6.0	5.5–6.0	5.2–5.6
Range	2.0–6.5	5.0–7.0	2.5–8.0	5.0–7.0	5.0–7.0	4.3–7.8
Temperature for growth (°C)						
Optimum	30–36	30–35	20–30	30–35	35–37	50–53
Range	20–45‡	15–42§	4–<45	15–37	15–42	34–65

*Growth with pyruvate.

†Growth with succinate or glutamate.

‡No growth at 15 or 50 °C.

§No growth at 10 or 45 °C.

||Weak growth at 4 °C and no growth at 45 °C.

et al., 2003; Johnson & Hallberg, 2005; Table 1), and other *Thiomonas* strains have been shown to oxidize and grow on carbon disulfide, dimethylsulfide and dimethyldisulfide (Pol *et al.*, 2007). The biotechnological application of *Thiomonas* strains for deodorization and bioremediation have also been recognized (Battaglia-Brunet *et al.*, 2003; Chen *et al.*, 2004; Pol *et al.*, 2007). Most strains show facultative chemolithoautotrophy and chemo-organotrophy, with optimum growth under mixotrophic conditions, but the autotrophic carbon-disulfide-oxidizing strain WZW appeared not to be facultatively chemo-organotrophic or mixotrophic (Pol *et al.*, 2007).

Emended description of *Thiomonas* Moreira and Amils 1997

Gram-negative, non-spore-forming, short rods that are about 0.3–0.9 µm wide and 1–4 µm long. Cells of most species are motile by means of a single polar flagellum.

Obligate aerobes. Optimum temperature 30–37 °C for mesophilic species and 50–53 °C for the moderately thermophilic species. Optimum pH between 3 and 6. The recognized species are facultative chemolithoautotrophs; optimum growth typically occurs in mixotrophic media supplemented with reduced sulfur compounds and organic supplements (yeast extract, peptone, some sugars or amino acids). Chemo-organotrophic growth is obtained on yeast extract, Casamino acids, peptone and meat extract. Most strains can grow chemolithoautotrophically with thiosulfate, tetrathionate, elemental sulfur or H₂S. Some strains can grow on carbon disulfide, dimethylsulfide or dimethyldisulfide as energy sources. Some strains oxidize ferrous iron [Fe(II)] and arsenite [As(III)], and some can oxidize and grow on metal sulfides, including chalcopyrite, arsenopyrite, sphalerite, galena and some synthetic sulfides. Sensitive to ampicillin. Major ubiquinone is Q-8. DNA G+C content is 61–69 mol%. Members of the *Betaproteobacteria*. The type species is *Thiomonas*

intermedia (London 1963) Moreira and Amils 1997 (= *Thiobacillus intermedius* London 1963).

Emended description of *Thiomonas cuprina* Moreira and Amils 1997

The emended description here is essentially that of '*Thiobacillus cuprinus*' given by Huber & Stetter (1990), as emended by Moreira & Amils (1997), Kelly & Wood (2005) and in this study. Cells are Gram-negative, aerobic rods, 1.0–4.0 µm long and 0.3–0.5 µm wide; motile with a single polar flagellum. Brownish-coloured colonies develop on complex media (yeast extract, peptone, Casamino acids, meat extract). Temperature optimum is 30–36 °C; no growth at 15 or 50 °C. Optimum pH for growth on yeast extract or sulfidic ores is pH 3.0–4.0, with a typical range of pH 2.0–6.5; no growth at pH 1.0 or 7.4. Some strains can grow on yeast extract or sulfide ores at pH 1.5–4.5. Facultative chemolithoautotrophs; aerobic. Chemo-organotrophic growth on yeast extract, peptone, Casamino acids and meat extract; some strains grow on pyruvate. No currently reported strains can grow on arabinose, fructose, galactose, glucose, lactose, mannose, raffinose, ribose, sorbose, sucrose, arginine, cysteine, glycine, L-glutamate, L-lysine, DL-serine, acetate, lactate, succinate, citrate or malate. Chemolithoautotrophic growth on a defined mixture of sulfide ores, chalcocite or arsenopyrite, or H₂S, and less efficiently on single sulfide ores (sphalerite, galena and synthetic CdS and FeS) or elemental sulfur. No growth on the natural ores bornite, chalcocite, covellite, pyrite, pitchblende or cinnabar or the synthetic sulfides Ag₂S, CuS, MoS, Sb₂S₃, SnS or ZnS. Ferrous sulfate is neither oxidized nor used as a growth substrate. Copper, zinc and uranium can be mobilized from ore mixtures and high levels of resistance are exhibited to cobalt, nickel and zinc, with significant tolerance of copper, arsenic and uranium. Sensitive to ampicillin. Contains ubiquinone Q-8 and *meso*-diaminopimelic acid, but not rusticyanin. Found in continental solfataric fields and mining environments.

The type strain is strain H5^T (=DSM 5495^T =NBRC 102145^T =NBRC 102094^T). The G + C content of the DNA of the type strain is 66.0 ± 0.6 mol% (*T_m*, HPLC). The 16S rRNA gene sequence of the type strain is deposited under GenBank/EMBL/DDBJ accession numbers AB331954 and AB331953.

REFERENCES

Altschul, S. F., Madden, T. L., Schäfer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database research programs. *Nucleic Acids Res* 25, 3389–3402.

Associate Editor, IJSB (1997). Notification that new names and new combinations have appeared in volume 47, no. 2, of the IJSB (p. 919, footnote d). *Int J Syst Bacteriol* 47, 917–919.

Battaglia-Brunet, F., Dictor, M.-C., Garrido, F., Crouzet, C., Morin, D., Dekeyser, K., Clarens, M. & Baranger, P. (2002). An arsenic(III)-

oxidizing bacterial population: selection, characterization, and performance in reactors. *J Appl Microbiol* 93, 656–667.

Battaglia-Brunet, F., Duquesne, K. F., Dictor, M.-C., Garrido, F., Bonnefoy, V., Baranger, P. & Morin, D. (2003). Arsenite oxidizing *Thiomonas* strains isolated from different mining sites. *Geophys Res Abstracts* 5, 11069.

Battaglia-Brunet, F., Joulain, C., Garrido, F., Dictor, M.-C., Morin, D., Coupland, K., Johnson, D. B., Hallberg, K. B. & Baranger, P. (2006). Oxidation of arsenite by *Thiomonas* strains and characterization of *Thiomonas arsenivorans* sp. nov. *Antonie van Leeuwenhoek* 89, 99–108.

Bruneel, O., Personné, J.-C., Casiot, C., Leblanc, M., Elbaz-Poulichet, F., Mahler, B. J., Le Flèche, A. & Grimont, P. A. D. (2003). Mediation of arsenic oxidation by *Thiomonas* sp. in acid-mine drainage (Carnoules, France). *J Appl Microbiol* 95, 492–499.

Chen, X.-G., Geng, A.-L., Yan, R., Gould, W. D., Ng, Y.-L. & Liang, D. T. (2004). Isolation and characterization of sulphur-oxidizing *Thiomonas* sp. and its potential application in biological deodorization. *Lett Appl Microbiol* 39, 495–503.

Huber, H. & Stetter, K. O. (1990). *Thiobacillus cuprinus* sp. nov., a novel facultatively organotrophic metal-mobilizing bacterium. *Appl Environ Microbiol* 56, 315–322.

Johnson, D. B. & Hallberg, K. B. (2005). Biogeochemistry of the compost bioreactor components of a composite acid mine drainage passive remediation system. *Sci Total Environ* 338, 81–93.

Katayama, Y., Uchino, Y., Wood, A. P. & Kelly, D. P. (2006). Confirmation of *Thiomonas delicata* (formerly *Thiobacillus delicatus*) as a distinct species of the genus *Thiomonas* Moreira and Amils 1997 with comments on some species currently assigned to the genus. *Int J Syst Evol Microbiol* 56, 2553–2557.

Katayama-Fujimura, Y. & Kuraishi, H. (1983). Emendation of *Thiobacillus perometabolis* London and Rittenberg 1967. *Int J Syst Bacteriol* 33, 650–651.

Katayama-Fujimura, Y., Enokizono, Y., Kaneko, T. & Kuraishi, H. (1983). Deoxyribonucleic acid homologies among species of the genus *Thiobacillus*. *J Gen Appl Microbiol* 29, 287–295.

Katayama-Fujimura, Y., Kawashima, I., Tzuzaki, N. & Kuraishi, H. (1984). Physiological characteristics of the facultatively chemolithotrophic *Thiobacillus* species *Thiobacillus delicatus* nom. rev., emend., *Thiobacillus perometabolis*, and *Thiobacillus intermedius*. *Int J Syst Bacteriol* 34, 139–144.

Kelly, D. P. & Harrison, A. H. (1989). Genus *Thiobacillus*. In *Bergey's Manual of Systematic Bacteriology*, vol. 3, pp. 1842–1858. Edited by J. T. Staley, M. P. Bryant, N. Pfennig & J. G. Holt. Baltimore: Williams & Wilkins.

Kelly, D. P. & Wood, A. P. (2005). Genus incertae sedis XVIII. *Thiomonas* Moreira and Amils 1997. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part C, pp. 757–759. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

Kelly, D. P., Wood, A. P. & Stackebrandt, E. (2005). Genus II. *Thiobacillus* Beijerinck 1904. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part C, pp. 764–769. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

London, J. (1963). *Thiobacillus intermedius* nov. sp., a novel type of facultative autotroph. *Arch Mikrobiol* 46, 329–337.

London, J. & Rittenberg, S. C. (1967). *Thiobacillus perometabolis* nov. sp., a non-autotrophic *Thiobacillus*. *Arch Mikrobiol* 59, 218–225.

Ludwig, W., Rossello-Mora, R., Aznar, R., Klugbauer, S., Spring, S., Reetz, K., Beimehr, C., Brockmann, E., Kirchhof, G. & other authors (1995). Comparative sequence analysis of 23S rRNA from *Proteobacteria*. *Syst Appl Microbiol* 18, 164–188.

Marin, I., Amils, R. & Abad, J. P. (1997). Genomic organization of the metal-mobilizing bacterium *Thiobacillus cuprinus*. *Gene* 187, 99–105.

Mizoguchi, T., Sato, T. & Okabe, T. (1976). New sulfur-oxidizing bacteria capable of growing heterotrophically, *Thiobacillus rubellus* nov. sp. and *Thiobacillus delicatus* nov. sp. *J Ferment Technol* **54**, 181–191.

Moreira, D. & Amils, R. (1996). PCR-mediated detection of the chemolithotrophic bacterium *Thiomonas cuprina* using 23S rDNA and 16S/23S intergenic spacer region-targeted oligonucleotide primers. *FEMS Microbiol Lett* **142**, 289–293.

Moreira, D. & Amils, R. (1997). Phylogeny of *Thiobacillus cuprinus* and other mixotrophic thiobacilli: proposal for *Thiomonas* gen. nov. *Int J Syst Bacteriol* **47**, 522–528.

Moreira, D., Amils, R. & Marin, I. (1994). Complete primary structure of the 23S rRNA coding gene from *Thiobacillus cuprinus* and its similarity with that of *Burkholderia cepacia*. *Syst Appl Microbiol* **17**, 481–483.

Pol, A., van der Drift, C. & Op den Camp, H. J. M. (2007). Isolation of a carbon disulfide utilizing *Thiomonas* sp. and its application in a biotrickling filter. *Appl Microbiol Biotechnol* **74**, 439–446.

Shooner, F., Bousquet, J. & Tyagi, R. D. (1996). Isolation, phenotypic characterization, and phylogenetic position of a novel, facultatively autotrophic, moderately thermophilic bacterium, *Thiobacillus thermosulfatus* sp. nov. *Int J Syst Bacteriol* **46**, 409–415.