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

Yoko Katayama,¹ Yoshihito Uchino,² Ann P. Wood³ and Donovan P. Kelly⁴

Correspondence

Donovan P. Kelly

D.P.Kelly@warwick.ac.uk

¹Department of Environmental and Natural Resource Science, Graduate School of Agriculture, Tokyo University of Agriculture and Technology, Fuchu, Tokyo 183-8509, Japan

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

³Department of Microbiology, King's College London Dental Institute at Guy's, King's College and St Thomas' Hospitals, Floor 28 Guy's Tower, Guy's Campus, London SE1 9RT, UK

⁴Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, UK

The transfer of *Thiobacillus delicatus* to the genus *Thiomonas* as a distinct species, *Thiomonas delicata* (type strain NBRC 14566^T), is confirmed by its morphological and physiological properties, DNA–DNA hybridization and the grouping of its 16S rRNA gene sequence with those of other species of the genus. An emended formal description of *Thiomonas delicata* is given. The status of *Thiomonas cuprina* DSM 5495^T as a member of the genus is reconsidered.

The genus *Thiomonas* was established by David Moreira and Ricardo Amils to comprise four species of chemolithotrophic autotrophs that formed a phylogenetic cluster within the β -1 subgroup of the *Betaproteobacteria* (Moreira & Amils, 1997). All four species (*Thiomonas intermedia*, *Thiomonas perometabolis*, *Thiomonas thermosulfata* and *Thiomonas cuprina*) were previously assigned to *Thiobacillus* but were found, by comparison of 5S and 16S rRNA gene sequences and by restriction fragment length analysis, to be rather remote from members of that genus (Moreira & Amils, 1997). Their closest phylogenetic neighbours within *Thiobacillus* were *Thiobacillus thioparus* and *Thiobacillus denitrificans*, which are in the β -2 subgroup of the *Betaproteobacteria* (Lane *et al.*, 1992). The 16S rRNA gene sequence similarity between *Thiobacillus thioparus* and *Thiomonas intermedia* was only 87%. Additional defining characteristics of the four *Thiomonas* species included the G + C content of their DNA being between 61 and 69 mol%, the presence of ubiquinone Q-8, the inability to denitrify nitrate to dinitrogen and optimum growth as mixotrophs when supplied with both a reduced inorganic sulfur compound and a variety of organic substrates, but the ability also to grow chemolithoautotrophically or as chemo-organotrophs (Katayama-Fujimura *et al.*, 1982, 1984; Moreira & Amils, 1997).

Moreira & Amils (1997) did not include *Thiobacillus delicatus* in the new genus as *Thiomonas delicata*, as insufficient phylogenetic data were then available, but they indicated that its affiliation with this mixotrophic group needed further investigation. *Thiobacillus delicatus* was originally isolated and characterized about 30 years ago (Mizoguchi *et al.*, 1976; Katayama-Fujimura *et al.*, 1982) and the name was formally revived with an emended species description by Katayama-Fujimura *et al.* (1984). It was retained in the list of species of the genus *Thiobacillus* in the 1989 and 2005 editions of *Bergey's Manual of Systematic Bacteriology* (Kelly & Harrison, 1989; Kelly *et al.*, 2005). However, Kelly & Wood (2005) proposed that *Thiobacillus delicatus* should also be transferred to the genus *Thiomonas* as *Thiomonas delicata*, on the basis of its physiological and biochemical properties (Table 1), subject to its phylogenetic relationship being confirmed by 16S rRNA gene sequencing (Kelly *et al.*, 2005; Kelly & Wood, 2005). This has now been done and we report that *Thiobacillus delicatus* should henceforth be known as *Thiomonas delicata* (Kelly & Wood, 2005, 2006).

A nearly complete 16S rRNA gene sequence (1456 nucleotides) of *Thiomonas delicata* strain THI 091^T (NBRC 14566^T) was determined at the NITE Biological Resource Center (NBRC) and was deposited with GenBank/EMBL/DBJ, under accession number AB245481. The sequence shared 93.4% similarity with that of the type species, *Thiomonas intermedia* (Table 1). Comparison with database

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of *Thiomonas delicata* NBRC 14566^T is AB245481.

Table 1. Comparison of *Thiomonas delicata* NBRC 14566^T with the type strains of other species of *Thiomonas*

Strains: 1, *Thiomonas delicata* NBRC 14566^T; 2, '*Thiomonas arsenivorans*' DSM 16361; 3, *Thiomonas intermedia* ATCC 15466^T; 4, *Thiomonas perometabolis* ATCC 23370^T; 5, *Thiomonas thermosulfata* ATCC 51520^T; 6, *Thiomonas cuprina* DSM 5495^T. Data were obtained from the present study and from London (1963), London & Rittenburg (1967), Mizoguchi *et al.* (1976), Huber & Stetter (1990), Katayama-Fujimura & Kuraishi (1983), Katayama-Fujimura *et al.* (1982, 1984), Kelly & Harrison (1989), Shooner *et al.* (1996), Moreira & Amils (1997), Battaglia-Brunet *et al.* (2006), Kelly & Wood (2005) and Kelly *et al.* (2005). NA, Data not available in the literature.

Characteristic	1	2	3	4	5	6
DNA G+C content (mol%)*	66–67 ^{a,b}	65 ^c	65–67 ^a	65–66 ^a	61 ^d	66–69 ^{a,c}
16S rRNA gene sequence similarity with <i>Thiomonas intermedia</i> (%)	93.4	93.5	(100)	99.7	94.1	87.3
DNA–DNA hybridization with <i>Thiomonas intermedia</i> DNA (%)	25–28	49	(100)	31–35	NA	NA
Cell width (µm)	0.4–0.6	0.3–0.5	0.6–0.8	0.4–0.5	0.9	0.3–0.5
Cell length (µm)	1.0–1.6	1.0–2.0	1.0–1.4	1.1–1.7	1.3–2.3	1.0–4.0
Motility	–	+	+	+	+	+
Flagellation	–	+	Polar	Polar	+	+
Ubiquinone	Q-8	NA	Q-8	Q-8	Q-8	Q-8
Major cellular fatty acids:†						
3-Hydroxydecanoic acid	+	NA	+	+	NA	NA
3-Hydroxy-8-methylnonanoic acid	+	NA	–	+	NA	NA
3-Hydroxydodecanoic acid	–	NA	+	+	NA	NA
Growth on/oxidation of:						
Thiosulfate	+	+	+	+	+	–
Tetrathionate	+	+	+	+	+	–
Fe(II)	NA	+	–	–	–	–
Arsenite	NA	+	+	–	NA	NA
Transitory formation of polythionates during thiosulfate oxidation	+	NA	–	–	+	–
Chemo-organotrophic growth with single organic compounds	–	+	–	+	+/-‡	+/-§
Nitrate reduction to nitrite	+	–	–	–	–	–
Optimum pH	5.5–6.0	4.0–7.5	5.5–6.0	5.5–6.0	5.2–5.6	3.0–4.0
pH limits for growth	5.0–7.0	2.5–8.0	5.0–7.0	5.0–7.0	4.3–7.8	2.0–6.5
Optimum temperature (°C)	30–35	20–30	30–35	35–37	50–53	30–36
Temperature limits for growth (°C)	15–42 ^a	4 to <45 ^b	15–37	15–42	34–65	– ^c

*G+C values were determined by using the following methods: ^athermal denaturation (T_m); ^bchemical analysis after hydrolysis with perchloric acid; ^cHPLC after enzymic digestion; ^dUV ratios (Ulitzur, 1972).

†*Thiomonas delicata*, *Thiomonas intermedia* and *Thiomonas perometabolis* contain hexadecanoic acid, hexadecenoic acid plus C₁₇ cyclopropane acid, and octadecenoic acid plus C₁₉ cyclopropane acid. NA for the other species.

‡Growth with succinate or glutamate.

§Growth with pyruvate.

||^aNo growth at 10 or 45 °C; ^bweak growth at 4 °C, but no growth at 45 °C; ^cNo growth at 15 or 50 °C.

sequences for other *Thiomonas* species using the BLAST algorithm (<http://www.ncbi.nlm.nih.gov/blast/>; Altschul *et al.*, 1997) showed 99.9% similarity of the *Thiomonas delicata* sequence with that of '*Thiomonas arsenivorans*' (Battaglia-Brunet *et al.*, 2006) and with those of *Thiomonas* sp. strain RCASK1 (AJ879998), *Thiomonas* sp. C19 (AF460989) and *Thiomonas* sp. CO2 (AF460988), with similarities of 93.4% with *Thiomonas intermedia* and *Thiomonas perometabolis*, 91.1% with *Thiomonas thermosulfata* and 88.8% with *Thiomonas cuprina* (Fig. 1).

The 16S rRNA gene sequence of *Thiomonas delicata* differs from that of '*Thiomonas arsenivorans*' and several other database sequences of *Thiomonas* spp. by only one to three nucleotides, but *Thiomonas delicata* and '*Thiomonas*

arsenivorans' are distinguished from each other by a number of physiological and biochemical characteristics (Table 1). In particular, *Thiomonas delicata* has the following properties that are not shared with '*Thiomonas arsenivorans*': it is non-motile, has more restricted ranges of temperature and pH for growth and optimum growth, can grow as an anaerobe using nitrate reduction to nitrite, but does not show heterotrophic growth on single organic compounds (glucose, fructose, ethanol, propanol, glycerol, oxalate, gluconate, pyruvate, lactate, malate, succinate, 2-oxoglutarate, citrate, alanine, serine, aspartate, glutamate, proline and histidine). Whereas '*Thiomonas arsenivorans*' grows autotrophically on arsenite and chemo-organotrophically on a wide range of organic acids and sugars (Battaglia-Brunet *et al.*, 2006), best growth of *Thiomonas*

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequences showing the relationship of *Thiomonas delicata* NBRC 14566^T to the other four recognized species of *Thiomonas* and '*Thiomonas arsenivorans*'. The analyses were performed using the neighbour-joining CLUSTAL X (version 1.82) method (Jeanmougin *et al.*, 1998) and evolutionary distances calculated by using the Kimura two-parameter procedure (Kimura, 1980). *Escherichia coli* (Gammaproteobacteria) was included as an outgroup. Bar, 2% nucleotide divergence. Numbers at nodes are bootstrap values from 1000 subsamples.

delicata is obtained on mixotrophic media with thiosulfate supplemented with Krebs cycle intermediates or amino acids. Optimal growth as a mixotroph is a property reportedly shared with *Thiomonas intermedia*, *Thiomonas perometabolis*, *Thiomonas thermosulfata* and *Thiomonas cuprina* (Moreira & Amils, 1997; Kelly & Wood, 2005). Whereas '*Thiomonas arsenivorans*' can grow well on yeast extract, *Thiomonas delicata* gave virtually no growth with 0.1% (w/v) yeast extract alone (about 5 mg cell protein l⁻¹), but supplementing with 0.5% (w/v) thiosulfate increased the biomass production to 90 mg cell protein l⁻¹, compared with about 40 mg cell protein l⁻¹ with thiosulfate alone (Mizoguchi *et al.*, 1976). With 0.5% (w/v) yeast extract, the biomass production was stimulated to about sixfold over that with thiosulfate alone (Katayama-Fujimura *et al.*, 1984). While these properties suggest that *Thiomonas delicata* and '*Thiomonas arsenivorans*' are separate species, determination of the ability of *Thiomonas delicata* to oxidize Fe(II) and As(III) and DNA–DNA

hybridization between them will help to establish whether '*Thiomonas arsenivorans*' is a distinct species or a subspecies of *Thiomonas delicata*. In this respect, it is noteworthy that the 16S rRNA gene sequences of *Thiomonas intermedia* and *Thiomonas perometabolis* also differ by only four nucleotides (99.7% similarity), but were shown by DNA–DNA hybridization, 23S rRNA gene and restriction fragment length polymorphism to be distinct species (Katayama-Fujimura *et al.*, 1982; Kelly & Harrison, 1989; Moreira & Amils, 1997).

The name *Thiomonas delicata* (Kelly & Wood, 2006) will have taxonomic precedence when validating the names of existing and future isolates of *Thiomonas* that are indistinguishable from it on the basis of their 16S rRNA gene sequences. Such isolates will require assessment of their physiological characteristics and comparative DNA–DNA hybridization with *Thiomonas delicata*. In this respect, there are numerous examples of species, in some cases from the same habitats, which share virtually identical 16S rRNA gene sequences (99–100%) but show only 0–35% interspecies DNA–DNA hybridization (Ash *et al.*, 1991; Fox *et al.*, 1992; Martinez-Murcia *et al.*, 1992; Jaspers & Overmann, 2004).

Thiomonas species can also exhibit extremely divergent 16S rRNA gene sequences, as is seen with *Thiomonas cuprina*, which shows only 85.9–88.8% sequence similarity with the other four *Thiomonas* species with validly published names. All five species have a rather distant relationship with strains of the β -1 betaproteobacterium *Burkholderia cepacia* (87–90% similarity) but, apart from *Thiomonas cuprina*, they show 91.1–99.7% similarity with each other. On the basis of physiological properties and molecular analyses, Moreira & Amils (1997) argued that *Thiomonas cuprina* should be included in the genus *Thiomonas*. However, the current wealth of betaproteobacterial 16S rRNA gene sequences indicates that *Thiomonas cuprina* is not securely placed as a recognized member of the *Thiomonas* clade on phylogenetic trees, being at least as closely related to *B. cepacia* and some other genera as to the *Thiomonas* cluster (Fig. 1; Y. Uchino, additional data not shown). None of the recognized species (or '*Thiomonas arsenivorans*'), except *Thiomonas cuprina*, has been reported to be capable of autotrophic growth on sulfide minerals. Moreira & Amils (1997) erroneously stated that *Thiomonas cuprina* also could not grow on sulfide minerals, but the type strain grew autotrophically on arsenopyrite, chalcopyrite, galena, cadmium sulfide and synthetic FeS (Huber & Stetter, 1990), although it did not oxidize Fe(II). In contrast to the other species of *Thiomonas* described to date, it could not grow on thiosulfate or tetrathionate as energy substrates, being able to use only more reduced sulfur substrates such as elemental sulfur and sulfides. With the exception of '*Thiobacillus plumbophilus*' (Drobner *et al.*, 1992), this inability to use thiosulfate is unique among all the species currently and previously known as *Thiobacillus* (Kelly & Harrison, 1989; Kelly *et al.*, 2005) and other chemolithotrophic thiosulfate-oxidizing *Proteobacteria* such as

Paracoccus, *Pseudaminobacter* and *Thiomicrospira*. However, *Thiomonas cuprina* and '*Thiobacillus plumbophilus*' are not closely related phylogenetically, as they show insignificant DNA–DNA hybridization (Drobner *et al.*, 1992) and share only about 85 % 16S rRNA gene sequence similarity (estimated by BLAST2 sequence comparison). Also, contrary to the description of *Thiomonas cuprina* provided by Moreira & Amils (1997), Huber & Stetter (1990) did not report that this species grew best under mixotrophic conditions. Unlike other *Thiomonas* species, the type strain of *Thiomonas cuprina* is moderately acidophilic with some strains being extreme acidophiles (Huber & Stetter, 1990). As well as sharing only 86–89 % 16S rRNA gene sequence similarity with the other *Thiomonas* species, *Thiomonas cuprina* shows only 85–89 % sequence similarity with other phylogenetically related *Betaproteobacteria*, including the type strains of *B. cepacia*, *Leptothrix discophora* and *Comamonas testosteroni*. *Thiomonas cuprina* also shares 87.5 % similarity of its 23S rRNA gene sequence with that of *B. cepacia* (Moreira & Amils, 1996). Currently, *Thiomonas cuprina* appears to have no closer phylogenetic neighbours than these genera (Moreira & Amils, 1997; D. P. Kelly, unpublished sequence analyses). It clearly differs from *B. cepacia* at the genus level (Selenska-Pobell *et al.*, 1998). There is thus a case for considering the promotion of *Thiomonas cuprina* as representing a novel genus of moderately acidophilic, metal sulfide-oxidizing, facultatively chemolithoautotrophic *Betaproteobacteria*.

The description of *Thiomonas delicata* is essentially that provisionally proposed by Kelly & Wood (2005), based on that of Katayama-Fujimura *et al.* (1984), as emended below. The type strain is available from the NBRC (Japan), the Korean Collection of Type Cultures (KCTC) and the DSMZ.

Emended description of *Thiomonas delicata* (Katayama-Fujimura *et al.* 1984) Kelly and Wood 2006

Thiomonas delicata (del.i.cat'a. L. fem. adj. *delicata* delicate).

Basonym: *Thiobacillus delicatus* (ex Mizoguchi *et al.* 1976) Katayama-Fujimura *et al.* 1984.

Rods, usually single, rarely in pairs, 0.4–0.6 µm wide and 1.0–1.6 µm long. Non-motile. Colonies grown on yeast extract-thiosulfate agar (1 mm in diameter) are smooth and circular and change from transparent to whitish-yellow with sulfur. Facultative chemolithotroph and mixotroph. Grows autotrophically with sulfur, thiosulfate and tetrathionate, but not with thiocyanate; accumulates tetrathionate and trithionate transiently during growth on thiosulfate. Incapable of chemo-organotrophic growth on single carbon compounds. Grows mixotrophically with thiosulfate as an energy source in mineral media supplemented with tricarboxylic acid cycle intermediates, amino acids or yeast extract. Optimum growth requires both organic substances and thiosulfate or sulfur. Facultative anaerobe; reduces nitrate and produces nitrite in mixotrophic and autotrophic

media with thiosulfate or tetrathionate, but does not denitrify nitrate to nitrogen gas. Ammonium salts, nitrate, urea, glutamate or aspartate can be used as a nitrogen source. Optimum temperature, 30–35 °C; growth range, 15–42 °C (no growth at 10 or 45 °C). Optimum pH, 5.5–6.0; growth range, pH 5.0–7.0. Isolated from mine water. Distribution unknown. The G + C content of the DNA is 66–67 mol% (T_m , chemical analysis).

The type strain is THI 091^T [= NBRC 14566^T (formerly IFO 14566^T) = KCTC 2851^T = DSM 17897^T]. The culture deposited as IAM 12624^T is no longer available from that collection.

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