NOTE

Reclassification of some species of *Thiobacillus* to the newly designated genera *Acidithiobacillus* gen. nov., *Halothiobacillus* gen. nov. and *Thermithiobacillus* gen. nov.

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The species of the genus '*Thiobacillus*' fall into the α-, β- and γ-subclasses of the *Proteobacteria*, the type species *Thiobacillus thioparus* being located in the β-subclass. '*Thiobacillus*' species exhibit almost as much diversity in DNA composition and physiology as is found collectively in all other proteobacterial groups. On the basis of physiological characters and 16S rRNA gene sequence comparisons, eight of the existing *Thiobacillus* species are proposed for reassignment to three newly designated genera within the γ-subclass of the *Proteobacteria*, namely *Acidithiobacillus*, *Halothiobacillus* and *Thermithiobacillus*.

Keywords: *Thiobacillus*, *Acidithiobacillus*, *Halothiobacillus*, *Thermithiobacillus*, reclassification

In the first edition of *Bergey's Manual of Systematic Bacteriology*, the heterogeneity of the genus *Thiobacillus* was emphasized, but insufficiently comprehensive information was available to enable a securely based reclassification of the species into new or existing genera (Kelly & Harrison, 1989). Since then, the widespread application of 16S rRNA gene sequence analysis and the use of DNA–DNA hybridization have provided tools for clarifying and dissecting the taxonomic infrastructure of the species currently classified as *Thiobacillus*, whose members fall into the α-, β- and γ-subclasses of the *Proteobacteria*, as shown in Fig. 1 (Woese *et al.*, 1984; Lane *et al.*, 1985, 1992; McDonald *et al.*, 1997; Goebel *et al.*, 1999). The application of these tools, together with traditional taxonomic devices, has already led to the transfer of six species from *Thiobacillus* into *Paracoccus* (the former *Thiobacillus versutus*; Katayama *et al.*, 1995; Rainey *et al.*, 1999), *Acidiphilium* (the former *Thiobacillus acidophilus*; Hiraishi *et al.*, 1998) and the new genus *Thiomonas* [the former *Thiobacillus intermedius*, *Thiobacillus perometabolis*, *Thiobacillus thermosulfatus* and '*Thiobacillus cuprinus*' (Moreira & Amils, 1997)]. We have assessed the phylogenetic diversity of the remaining species and shown how some form acceptable taxonomic groupings (Kelly *et al.*, 1998; McDonald *et al.*, 1997).

In writing the *Thiobacillus* section for the second edition of *Bergey's Manual of Systematic Bacteriology*, we (Kelly & Wood, 2000) have reassigned eight further species to three new genera. These genera are *Acidithiobacillus* (containing *Thiobacillus thiooxidans*, *Thiobacillus ferrooxidans*, *Thiobacillus caldus* and *Thiobacillus albertensis*), *Halothiobacillus* (containing *Thiobacillus neapolitanus*, *Thiobacillus halophilus* and *Thiobacillus hydrothermalis*) and *Thermithiobacillus* (containing *Thiobacillus tepidarius*). In Fig. 1, we illustrate the locations of the principal members of these new genera within the subclasses of the *Proteobacteria*. This reassignment and creation of appropriate genera was encouraged by the editors of the *Manual*; validation by IJSB/IJSEM of names published in *Bergey's Manual of Systematic Bacteriology* is normal practice (J. T. Staley, personal communication). It is the purpose of this paper to present, formally, the new genus designations and new species combinations proposed in the *Manual*.

Status of the genus *Thiobacillus* Beijerinck (1904)

The currently recognized species of *Thiobacillus* exhibit a wide range of physical growth conditions, e.g. tolerance of pH values from around 0 to above 8·5 (with pH and temperature optima of < 2–8 and 20–50 °C, respectively), diverse G + C content of the DNA (50–68 mol%), diversity of DNA homology and a range of ubiquinones and fatty acids. All are small, Gram-negative, rod-shaped bacteria (0·3–0·5 × 0·7–4·0 μm), some species being motile by means of polar

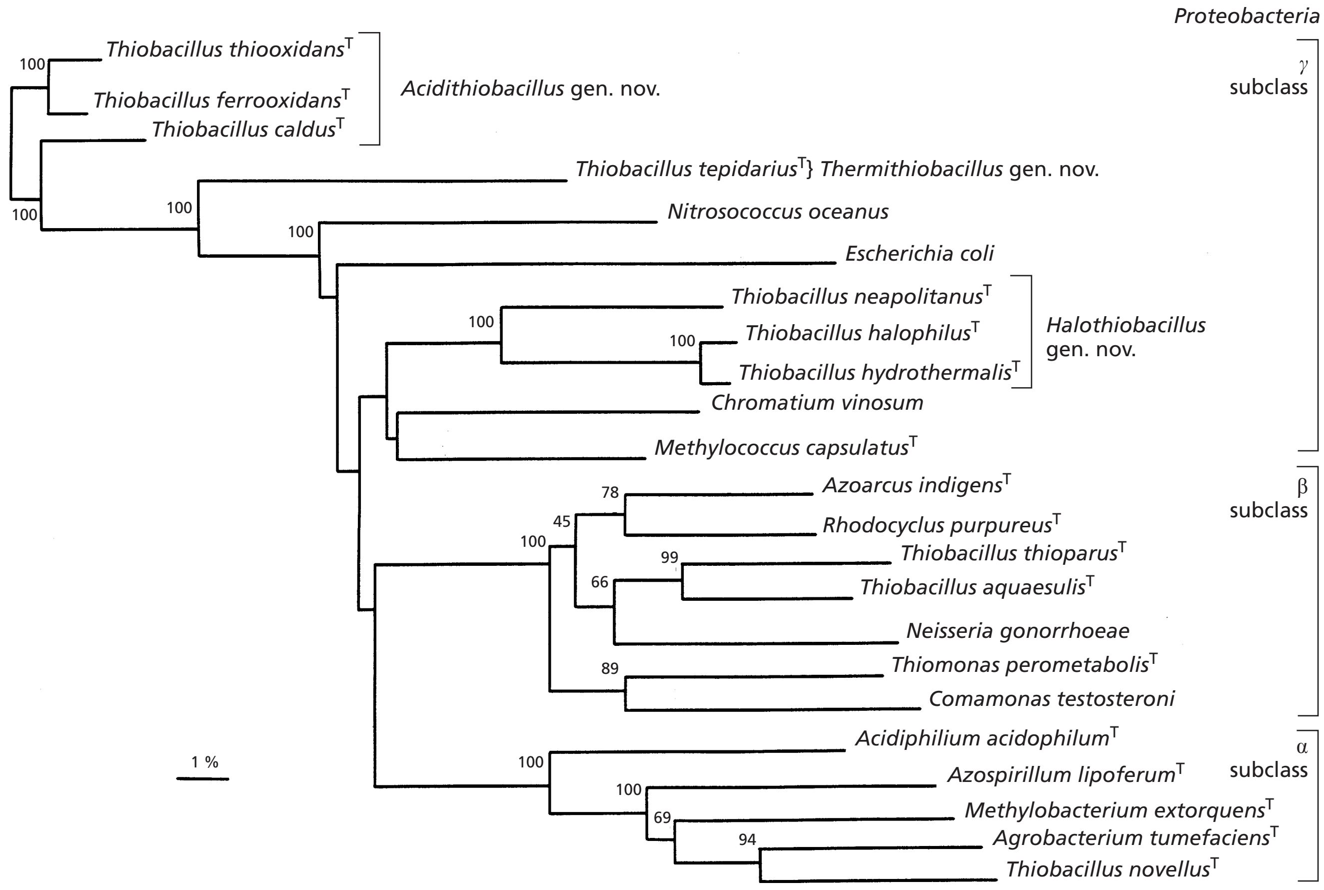

Fig. 1. Phylogenetic tree based on 16S rRNA gene-sequence data analysis of members of the *Proteobacteria*, showing '*Thiobacillus*' species in each of the α-, β- and γ-subclasses. The type species (*Thiobacillus thioparus*) is located in the β-subclass. The new genus designations for seven of the *Thiobacillus* species are also shown. Note that *Thiomonas perometabolis* and *Acidiphilium acidophilum* were originally also described as *Thiobacillus* species. The dendrogram shows the results from analyses using DNADIST, giving the bootstrap values (McDonald *et al.*, 1997) from 100 replicates. Bar, 1 % sequence divergence, determined by comparing the lengths of the horizontal lines connecting any two species. T, Type strain.

flagella; no resting stages are known. Energy is derived from the oxidation of one or more reduced sulfur compounds, including sulfides, sulfur, thiosulfate, polythionates and thiocyanate. Sulfate is the end product of sulfur-compound oxidation, but sulfur, sulfite or polythionates may be accumulated, sometimes transiently, by most species. Some species also derive energy by oxidizing organosulfur compounds or by oxidizing ferrous iron to ferric iron. All species can fix carbon dioxide by means of the Benson–Calvin cycle and are capable of autotrophic growth; some species are obligately chemolithotrophic, while others are also able to grow chemo-organotrophically. The genus currently includes obligate aerobes and facultative denitrifying types. This diversity of properties among species is indicative of an extremely heterogeneous group, judged in terms of genetic and physiological similarity. Indeed, the only historical criterion for concentrating all the species into one genus was that all are rod-shaped eubacteria able to obtain energy for autotrophic growth by oxidizing inorganic sulfur substrates. Their distribution is seemingly ubiquitous across marine, freshwater and soil environments, especially where oxidizable sulfur is abundant (e.g. sulfur springs, sulfide minerals, sulfur deposits, sewage-treatment areas and sources of sulfur gases, such as H_2S from sediments or anaerobic soils). Analysis of 16S rRNA sequences has revealed a diversity and misclassification among the thiobacilli at least as profound as that shown many years ago, by classical means, for the 'hydrogen bacteria', which were subsequently reclassified into diverse genera (Davis *et al.*, 1969).

The genus *Thiobacillus* after revision in 1999

The type species, *Thiobacillus thioparus* (Beijerinck, 1904), is a member of the β-subclass of the *Proteobacteria*, so the original genus and species name must be retained for this organism. Other members of the β-subclass currently retained as *Thiobacillus* are *Thiobacillus denitrificans*, which is very closely related to the type species, and *Thiobacillus aquaesulis* (Fig. 1) and '*Thiobacillus plumbophilus*'. '*Thiobacillus plumbophilus*' (Drobner *et al.*, 1992) has never been validated and is in need of more detailed comparative study to determine if its retention as a *Thiobacillus* species is justified. *Thiobacillus novellus*, in the α-subclass, and '*Thiobacillus prosperus*' (Huber & Stetter, 1989), in the γ-subclass, have not yet been removed from the genus as insufficient data are currently available to assign them to existing or new genera (Kelly & Wood, 2000). Similarly, *Thiobacillus delicatus* (Katayama-Fujimura *et al.*, 1984) currently remains in the *Thiobacillus* genus (as its 16S rRNA sequence has not been reported) but its mixotrophy and physiological similarities to *Thiomonas perometabolis* indicate that it should probably be assigned to the *Thiomonas* genus (Katayama-Fujimura *et al.*, 1984; Moreira & Amils, 1997).

Assignment of some species of *Thiobacillus* to new genera

The identity and properties of the species being reassigned are identified in Table 1. The species (all of which are members of the γ-subclass of the *Proteobacteria*) are seen to be broadly divided into three groups. One group comprises a solitary species, *Thiobacillus tepidarius*; of the two larger groups, one comprises the proposed new genus, *Halothiobacillus*, and the other comprises *Acidithiobacillus*, containing four of the acidophilic species, some of which also oxidize iron[II] and sulfide minerals (Table 1; McDonald *et al.*, 1997; Kelly *et al.*, 1998; Kelly & Wood, 2000). The acidophilic and strictly chemolithotrophic *Thiobacillus albertensis* is included in *Acidithiobacillus* genus, being physiologically similar to *Thiobacillus thiooxidans*, but is distinguished by possessing a glycocalyx and being motile by means of a tuft of flagella, suggesting that its taxonomic status requires further study. The name of this species is also now corrected to *Acidithiobacillus albertensis* (see the species description below). *Thiobacillus tepidarius* seems somewhat distantly related to this group (McDonald *et al.*, 1997; Goebel *et al.*, 1999), but we currently consider its moderate thermophily and lack of acidophily sufficient grounds to regard it as a species of a distinct genus (Kelly & Wood, 2000). Comparative 16S rRNA similarity values for all these species (except *Thiobacillus albertensis*) have been published elsewhere (McDonald *et al.*, 1997).

Description of *Acidithiobacillus* (Kelly and Wood 2000) gen. nov.

Acidithiobacillus (A.ci.di′thi.o.ba.cil′lus. L. adj. *acidus* sour; Gr. n. *thios* sulfur; L. n. *bacillus* a small rod; M.L. masc. n. *Acidithiobacillus* acid-loving sulfur rodlet).

Obligately acidophilic (optimum pH $< 4{\cdot}0$) and aerobic, Gram-negative rods ($0{\cdot}4 \times 2{\cdot}0$ µm), motile by one or more flagella, using reduced sulfur compounds to support autotrophic growth. Some species oxidize ferrous iron or use natural and synthetic metal sulfides for energy generation; some species oxidize hydrogen. Optimum temperature 30–35 °C for mesophilic species and 45 °C for moderately thermophilic species. Contain ubiquinone Q-8. The G + C content of the DNA is 52–64 mol%. Other general characteristics are those of *Thiobacillus*. Members of the γ-subclass of the *Proteobacteria*. The type species is *Acidithiobacillus thiooxidans* (formerly *Thiobacillus thiooxidans*).

Description of *Acidithiobacillus thiooxidans* (Waksman and Joffe 1922) comb. nov.

Acidithiobacillus thiooxidans (thio.ox′i.dans. Gr. n. *thios* sulfur; M.L. v. *oxido* make acid, oxidize; M.L. part. adj. *thiooxidans* sulfur-oxidizing).

The description of *Acidithiobacillus thiooxidans* is given by Waksman & Joffe (1922), Kelly & Harrison (1989) and Kelly & Wood (2000). It is motile by means of a polar flagellum (Doetsch *et al.*, 1967). It grows in liquid medium on elemental sulfur, thiosulfate or tetrathionate; it cannot oxidize iron or pyrite but has been shown to grow on sulfur from pyrite in co-culture with '*Leptospirillum ferrooxidans*', an iron-oxidizing, sulfur-non-oxidizing vibrio. The 16S rRNA sequence accession numbers are M79396–98 and M79401, and the 5S rRNA sequence accession number is M11541. The type strain is ATCC 19377^{T} (= NCIMB 8343^{T}).

Taxonomic note. *Thiobacillus concretivorus* (Parker, 1945) was once listed as a distinct species in *Bergey's Manual of Determinative Bacteriology* (Parker, 1957) and appeared in the *Approved Lists of Bacterial Names* (Skerman *et al.*, 1980). It is now recognized, however, as a later synonym of *Thiobacillus thiooxidans* (Vishniac, 1974; Kelly & Harrison, 1989) and should be deleted from the Approved Lists.

Description of *Acidithiobacillus ferrooxidans* (Temple and Colmer 1951) comb. nov.

Acidithiobacillus ferrooxidans (ferro.ox′i.dans. L. n. *ferrum* iron; *ferro-* denoting the +2 (ferrous) oxidation state of the iron; M.L. v. *oxido* oxidize, make acid; M.L. part. adj. *ferrooxidans* ferrous-iron-oxidizing).

The description of *Acidithiobacillus ferrooxidans* is given by Temple & Colmer (1951), Kelly & Harrison (1989) and Kelly & Wood (2000). The accession numbers for the 16S rRNA sequence are M79404–6, and the 5S rRNA sequence accession number is M11542. The type strain is ATCC 23270^{T}.

Taxonomic note. Many strains of *Acidithiobacillus ferrooxidans* have been described in the literature, all sharing the more-or-less uniform phenotype of being acidophilic, obligately chemolithoautotrophic, Gram-negative rods that use the oxidation of ferrous iron for energy generation. They do, however, exhibit considerable genetic variation (Harrison, 1982; Kelly &

Table 1. Key features of the type strains of the obligately chemolithoautotrophic *Thiobacillus* species assigned to the new genera *Acidithiobacillus*, *Halothiobacillus* and *Thermithiobacillus*

New name combination	Culture collection no.	Nucleotide sequence accession no.	DNA G+C content (mol %)	Conditions for optimum chemolithoautotrophic growth (limits in parentheses)			Lowest pH after growth on sulfur compounds	Other special properties
				Temp (°C)	pH	NaCl (M)		
Acidithiobacillus thiooxidans**	ATCC 19377^{T} NCIB 8347^{T}	M79396–8 M79401	52	28–30 (10–37)	2·0–3·0 (0·5–5·5)	0‡	0·5–0·8	
Acidithiobacillus ferrooxidans	ATCC 23270^{T}	M79404–6	58–59	30–35 (10–37)	2·5 (1·3–4·5)	0	1·5	Growth with Fe[II] as sole energy substrate
Acidithiobacillus caldus	DSM 8584^{T}	Z29975	63·1–63·9	45 (32–52)	2·0–2·5 (1·0–3·5)	0	NA†	
Acidithiobacillus albertensis	ATCC 35403^{T}	NA†	61·5	25–30	3·5–4·0 (2·0–4·5)	0	2·0	Glycocalyx and tuft of flagella
*Halothiobacillus neopolitanus**	NCIB 8539^{T}	M79399 M79419–20	56	28–32 (8–39)	6·5–6·9 (4·5–8·5)	ST§	2·8–3·3	
Halothiobacillus halophilus	DSM 6132^{T} ATCC 49870^{T}	U58020	64·2	30–32 (26–36)	7·0–7·3 (6·5–8·4)	1·0 (4·0)	5·5–6·0	Obligate halophile
Halothiobacillus hydrothermalis	DSM 7121^{T}	M90662	67·1–67·4	35–40 (11–48)	7·5–8·0 (5·5–9·0)	0·43 (0–2·0)	4·8	Poor growth without NaCl
*Thermithiobacillus tepidarius**	DSM 3134^{T} ATCC 43215^{T}	M79424–5	66·6	43–45 (20–52)	6·8–7·5 (5·5–8·0)	0	4·5–5·0	

* Type species of genus.

† Values not available.

‡ No NaCl requirement.

§ Solute tolerant (e.g. 6 %, w/v, sodium thiosulfate).

Harrison, 1989; Goebel *et al.*, 1999). Using PCR-based techniques to assess this genomic variability, similarity coefficients between various isolates were obtained which ranged from almost 0 % to over 98 % (Novo *et al.*, 1996); while several culture-collection isolates were very closely related to the type strain (ATCC 23270^{T}), one strain (ATCC 33020) was not taxonomically related to the others (Selenska-Pobell *et al.*, 1998). It is inevitable, therefore, that some strains assigned to this species will be reassigned to new species or genera in due course.

Description of *Acidithiobacillus caldus* (Hallberg and Lindström 1994) comb. nov.

Acidithiobacillus caldus (cal′dus. M.L. adj. *caldus* warm, liking warmth).

The description of *Acidithiobacillus caldus* is given by Hallberg & Lindström (1994), Kelly & Harrison (1989) and Kelly & Wood (2000). It shows no significant DNA hybridization (2–20 %) with *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Thiomonas cuprina* or *Thiobacillus thioparus*. The accession number for the 16S rRNA sequence is Z29975. The type strain is DSM 8584^{T}.

Description of *Acidithiobacillus albertensis* (Bryant, McGroarty, Costerton and Laishley 1988) nom. corrig., comb. nov.

Acidithiobacillus albertensis (al.ber.ten′sis. M.L. adj. *albertensis* Albertan, pertaining to Alberta, a province of Canada).

The description of *Acidithiobacillus albertensis* is given by Bryant *et al.* (1983, 1988), Kelly & Harrison (1989) and Kelly & Wood (2000). The type strain is ATCC 35403^{T}. The suggestion of assigning *Thiobacillus albertensis* to the new genus *Acidithiobacillus* is tentative, as its 16S rRNA sequence does not appear to be available yet. The relatively high G+C content of its DNA, together with its possession of a tuft of flagella and a glycocalyx, constitute significant differences from the other three species included here. Unfortunately, the original isolate may have been lost from culture (B. M. Goebel, personal communication).

Description of *Halothiobacillus* gen. nov.

Halothiobacillus (Hal.o.thi.o.ba.cil′lus. Gr. n. *hals* sea, salt; Gr. n. *thios* sulfur; L. n. *bacillus* a small rod; L. masc. n. *Halothiobacillus* salt-loving sulfur rodlet).

Obligately chemolithoautotrophic Gram-negative rods, 0·3–0·6 × 1·0–1·5 µm, obtaining energy from reduced inorganic sulfur compounds. Tolerant of high concentrations of solutes (e.g. 4 M NaCl; 0·25 M sodium thiosulfate), with some strains showing moderate halophily with a requirement for NaCl. Unable to oxidize thiocyanate or ferrous iron. Optimum temperature 30–40 °C and optimum pH 6·5–8·0. Contain ubiquinone Q-8. The G + C content of the DNA is 56–68 mol %. Other general characteristics are those of *Thiobacillus* (Wood & Kelly, 1988; Kelly & Harrison, 1989; Kelly & Wood, 2000). Members of the γ-subclass of the *Proteobacteria*. The type species is *Halothiobacillus neapolitanus* (formerly *Thiobacillus neapolitanus*). Other distinctive features of the species of *Halothiobacillus* are described elsewhere (Kelly *et al*., 1998; Kelly & Wood, 2000).

Description of *Halothiobacillus neapolitanus* (Parker 1957) comb. nov.

Halothiobacillus neapolitanus (ne.a.po.li.ta′nus. L. adj. *neapolitanus* Neapolitan; pertaining to the sea water at Naples from which this species was probably first isolated by Nathansohn in 1902).

The description of *Halothiobacillus neapolitanus* comb. nov. is that given by Parker (1957) for *Thiobacillus* strain X, as updated by Kelly & Harrison (1989) and Kelly & Wood (2000). The 16S rRNA accession numbers are M79399, M79419 and M79420. The type strain is strain NCIMB 8539^{T} (= DSM 581^{T}).

Description of *Halothiobacillus halophilus* (Wood and Kelly 1991) comb. nov.

Halothiobacillus halophilus (hal.o′phil.us. Gr. n. *hals* salt; Gr. adj. *philos* loving; M.L. adj. *halophilus* salt-loving).

The description of *Halothiobacillus halophilus* comb. nov. is that given by Wood & Kelly (1991) and Kelly & Wood (2000). The 16S rRNA accession number is U58020. The type strain is strain DSM 6132^{T} (= ATCC 49870^{T}).

Description of *Halothiobacillus hydrothermalis* (Durand, Reysenbach, Prieur and Pace 1993) comb. nov.

Halothiobacillus hydrothermalis (hy.dro.ther.mal′is M.L. adj. *hydrothermalis* pertaining to a hydrothermal vent in the North Fiji basin).

The description of *Halothiobacillus hydrothermalis* comb. nov. is given by Durand *et al*. (1993) and Kelly & Wood (2000). The 16S rRNA sequence accession number is M90662. The type strain is strain DSM 7121^{T}.

Description of *Thermithiobacillus* (Kelly and Wood 2000) gen. nov.

Thermithiobacillus (Therm.i.thi.o.ba.cil′lus. L. fem. pl. n. *thermae* warm baths; Gr. n. *thios* sulfur; L. n. *bacillus* a small rod; L. masc. n. *Thermithiobacillus* warmth-loving sulfur rodlet).

Obligately aerobic, moderately thermophilic and chemolithoautotrophic, Gram-negative, non-spore-forming, rods (0·5 × 1–2 µm). Grow on reduced inorganic sulfur compounds; unable to oxidize ferrous iron. Contain ubiquinone Q-8; the G + C content of the DNA is about 66–67 mol %. Other general characteristics are those of *Thiobacillus*. Members of the γ-subclass of the *Proteobacteria*. The type species is *Thermithiobacillus tepidarius* (formerly *Thiobacillus tepidarius*).

Description of *Thermithiobacillus tepidarius* (Wood and Kelly 1985) comb. nov.

Thermithiobacillus tepidarius (tep.i.dar′ius. L. n. *tepidarium* a warm bath fed by natural thermal water; M.L. adj. *tepidarius* warm-bathing).

The description of *Thermithiobacillus tepidarius* is that given in Wood & Kelly (1985, 1986) and Kelly & Wood (2000). The 16S rRNA sequence accession numbers are M79424 and M79425. The type strain is DSM 3134^{T} (= ATCC 43215^{T}).

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