NOTE

Proposal for the reclassification of *Thiobacillus novellus* as *Starkeya novella* gen. nov., comb. nov., in the α -subclass of the *Proteobacteria*Donovan P. Kelly,¹ Ian R. McDonald¹ and Ann P. Wood²

Author for correspondence: Donovan P. Kelly. Tel: +44 24 7657 2907. Fax: +44 24 7652 3701.
e-mail: dkelly@cell.bio.warwick.ac.uk

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

² Microbiology Research Group, Division of Life Sciences, King's College London, Franklin-Wilkins Building, 150 Stamford Street, London SE1 8WA, UK

***Thiobacillus novellus* is a facultatively chemolithoautotrophic and methylophilic, Gram-negative, rod-shaped sulfur bacterium, shown by 16S rRNA gene sequence analysis to be a member of the α -2 subclass of the *Proteobacteria*. As such, it must be excluded from the genus *Thiobacillus*, whose species are members of the β -*Proteobacteria*. Its closest phylogenetic neighbour appears to be *Ancylobacter*, from which it is distinct morphologically and in some physiological characteristics. It is distinct physiologically and biochemically in a number of diagnostic features from *Paracoccus versutus*, in the α -3 subclass of the *Proteobacteria* and does not appear to be sufficiently closely related to any other genus of the α -*Proteobacteria* to be reassigned to a known genus. The new genus and species name *Starkeya novella* is proposed for *T. novellus*. The type strain is ATCC 8093^T (= NCIMB 10456^T = NCIMB 9113^T = DSM 506^T = IAM 12100^T = IFO 12443^T = CCM 1077^T).**

Keywords: *Thiobacillus novellus* ATCC 8093^T, *Starkeya novella* ATCC 8093^T, 16S rRNA phylogeny

Thiobacillus novellus was isolated by Starkey (1934, 1935a, b) and described as the first facultatively heterotrophic *Thiobacillus* species to be discovered that is able to grow either chemolithoautotrophically with thiosulfate as an energy source or heterotrophically on some organic media (Vishniac & Santer, 1957; Kelly & Harrison, 1989). Subsequently, Taylor & Hoare (1969) isolated another facultative strain, which was at first regarded as a biotype of *T. novellus* but was later classified as a new species, *Thiobacillus versutus* (Harrison, 1983), before being reassigned as a distinct species of the genus *Paracoccus* (Katayama *et al.*, 1995; Rainey *et al.*, 1999). Considerable work has been published on the physiology of *T. novellus*, but its taxonomic status has never been definitively clarified, although the numerical taxonomy study of Hutchinson *et al.* (1965, 1969) clearly showed it to be distinct from the other *Thiobacillus* groups identified. We now summarize the evidence supporting the view that *T. novellus* can no longer be retained as a species of *Thiobacillus* and that it is not a species of *Paracoccus*.

We propose its assignment to a new genus, *Starkeya*, as *Starkeya novella*.

Numerous species of the original *Thiobacillus* genus have been assigned to new or different genera (Moreira & Amils, 1997; Hiraishi *et al.*, 1998; Kelly & Wood, 2000a; Kelly *et al.*, 2000), with only some species, located in the β -subclass of the *Proteobacteria*, being retained as true species of *Thiobacillus*. These include the type species *Thiobacillus thioparus*, as well as *Thiobacillus denitrificans*, *Thiobacillus aquaesulis* and (at present) *Thiobacillus plumbophilus* (McDonald *et al.*, 1997; Kelly & Wood, 2000a, b; Kelly *et al.*, 2000). As *T. novellus* has been shown by 16S rRNA gene sequence analysis and by analysis of polyamine content to be a member of the α -subclass of the *Proteobacteria* (Hamana & Matsuzaki, 1990; McDonald *et al.*, 1997), it must be excluded from the genus *Thiobacillus*. Two facultatively heterotrophic *Thiobacillus*-like species in the α -subclass, *T. versutus* and *Thiosphaera pantotropha*, have been reassigned to the genus *Paracoccus* (Rainey *et al.*, 1999). Sijderius (1946) considered that *T. novellus* was also a species of *Micrococcus* (now *Paracoccus*) and that it should be renamed *Micrococcus denitrificans* var. *Starkeyi*. *T. novellus* is, how-

The GenBank/EMBL/DBJ accession number for the 16S rRNA sequence of *Starkeya novella* is D32247.

Fig. 1. Phylogenetic tree based on 16S rRNA gene sequence data analysis of *Starkeya novella* (formerly *Thiobacillus novellus*) and other α -Proteobacteria, using published 16S rRNA sequences. Bootstrap values from 100 replicates are also shown. Bar, 10% sequence divergence, as determined by measurement of the lengths of the horizontal lines connecting any two species. The 16S rRNA sequence of *Escherichia coli* strain ATCC 11775 was used to root the tree. The database accession numbers of the 16S rRNA sequences used to construct the tree are as follows: *Agrobacterium tumefaciens*, M11223; *Paracoccus thiocyanatus*, D32242; *Paracoccus denitrificans*, Y16927; *Paracoccus pantotrophus*, Y16933; *Paracoccus versutus*, Y16932; *Rhodoplanes elegans*, D25311; *Rhodoplanes roseus*, D25313; *Ancylobacter aquaticus*, M62790; *Starkeya novella*, D32247; *Azorhizobium caulinodans*, D11342; *Xanthobacter flavus*, X94202; *Rhodopseudomonas palustris*, X87279; *Bradyrhizobium japonicum*, X87272; *Methylobacterium organophilum*, D32226; *Methylobacterium rhodinum*, D32229; *Methylobacterium extorquens*, D32224; *Methylobacterium rhodesianum*, D32228.

ever, located in the α -2 subclass of the *Proteobacteria*, which also contains *Ancylobacter*, *Methylobacterium*, *Xanthobacter* and *Azorhizobium* (Fig. 1; Hamana & Matsuzaki, 1990; Hiraishi *et al.*, 1995; Rainey & Wiegand, 1996; Holmes *et al.*, 1997; Stubner *et al.*, 1998), but appears from 16S rRNA sequence analysis to be only distantly related to *Paracoccus* species, which fall into the α -3 subclass (Fig. 1; Katayama *et al.*, 1995). In the earlier years of molecular taxonomy, Lane *et al.* (1985) concluded from a comparison of their 5S rRNA sequences that '*Thiobacillus novellus* may merit its own subgroup, but it clearly belongs to a

different subgroup from *Thiobacillus versutus*'. The two species shared only approximately 71% identity in 115 5S rRNA bases, as estimated from Lane *et al.* (1985), who, however, had insufficient data to place it phylogenetically.

Comparison of the physiological properties of *T. novellus* and *Paracoccus* shows that *T. novellus* should not be classified as a species of *Paracoccus*. It differs from *Paracoccus versutus* in growth-substrate range, vitamin requirement, polyamine content, motility, denitrifying ability, DNA-DNA hybridization and LPS and fatty acid content (Table 1). Thiosulfate oxidation by *T. novellus* is effected by a membrane-associated complex, the enzymes of which are tightly bound within the membrane structure (Oh & Suzuki, 1977a, b). This contrasts with the case of *P. versutus*, in which the thiosulfate-oxidizing multienzyme system is located in the periplasm and does not require the membrane system for activity *in vitro* (Lu & Kelly, 1983; Lu *et al.*, 1985; Kelly, 1989; Kelly *et al.*, 1997). While *P. versutus* is unable to oxidize tetrathionate as a source of energy, *T. novellus* can use this as a substrate, although it cannot grow on elemental sulfur, which is a substrate for *P. versutus* (Charles & Suzuki, 1966; Taylor & Hoare, 1969; Feldmann & Goroll, 1976; Katayama-Fujimura & Kuraishi, 1980; Beffa *et al.*, 1993). Most studies of *T. novellus* have used the type strain (ATCC 8093^T) or cultures derived from it (e.g. NCIMB 10456^T and IAM 12100^T), but one report of a new isolate demonstrated both oxidation of thiosulfate to tetrathionate, a reaction not catalysed by *Paracoccus*, and the ability to oxidize methanethiol, dimethylsulfide and dimethyldisulfide, which are not substrates for *P. versutus* (Cha *et al.*, 1999). If confirmed for the type strain, this would be an additional difference between *T. novellus* and *P. versutus*.

The glucose-dissimilation mechanisms in *T. novellus* and *P. versutus* are completely different. *P. versutus* simultaneously employs the Embden-Meyerhof and Entner-Doudoroff pathways and an oxidative pentose pathway for glucose oxidation (Wood & Kelly, 1977, 1978, 1980; Wood *et al.*, 1977), but *T. novellus* lacks the Embden-Meyerhof and Entner-Doudoroff pathways and employs a non-cyclic phosphoketolase-dependent pentose phosphate pathway (Table 1; Matin & Rittenberg, 1971; Greenley & Smith, 1979).

Autotrophic growth on formate, using ribulose 1,5-bisphosphate carboxylase, is common to both *T. novellus* and *Paracoccus denitrificans* (Chandra & Shethna, 1977; Kelly *et al.*, 1979). In contrast, *T. novellus* contained only low levels of this carboxylase during growth on methanol and it was suggested that it uses the ribulose monophosphate cycle of formaldehyde fixation for growth on methanol (Chandra & Shethna, 1977), while methanol assimilation by *P. versutus* is autotrophic (Kelly & Wood, 1982).

Comparison of 16S rRNA gene sequences revealed a number of genera showing greater phylogenetic

Table 1. Comparison of *Thiobacillus novellus*, *Paracoccus versutus*, *Ancylobacter aquaticus*, *Xanthobacter* spp. and *Azorhizobium caulinodans*

Characteristic	<i>T. novellus</i>	<i>P. versutus</i>	<i>A. aquaticus</i>	<i>Xanthobacter</i> spp.	<i>A. caulinodans</i>
DNA G + C content (mol %)	67.3–68.4	67–69	66.3–67.7	67–69	66–68
Proteobacterial subclass (16S rRNA)	α -2	α -3	α -2	α -2	α -2
DNA–DNA hybridization with <i>T. novellus</i> DNA (%)	100	0–14	NA	NA	NA
Root/stem nodule symbiont	–	–	–	–	+
Yellow colony pigmentation	–	–	–	+	–
Gas vacuoles produced	–	–	+	–	–
Cell morphology					
Rods or coccobacilli	+	+	–	+	+
Curved/vibrioid rods, rings	–	–	+	–	–
Pleomorphism	–	–	–	+	–
Motility	–	+	–	+	+
Plasmid/megaplasmid present	–	+	+	NA	NA
Ubiquinone	Q ₁₀	Q ₁₀	Q ₁₀	Q ₁₀	NA
Major cellular fatty acids					
Octadecenoic acid (C _{18:1})	+	+	+	+	NA
Cyclopropane acid of C ₁₉	+	+	–	NA	NA
Cellular hydroxy-fatty acid	None	3-OH-C _{10:0}	3-OH-C _{10:0}	3-OH-C _{16:0}	NA
Polyamine type					
Putrescine	+	+	NA	NA	+
Spermidine	–	+	NA	NA	–
Homospermidine	+	–	NA	NA	+
Growth on/oxidation of:					
Thiosulfate	+	+	+	+	NA
Tetrathionate	+	–	+	+	NA
Sulfur	–	+	NA	NA	NA
Thiocyanate	–	–	NA	NA	NA
Growth on:					
Formate	+	+	+	+	–
Methanol	+	+	+	+	–
Methylamine	–	+	+	+	–
Oxalate	+	–	NA	NA	–
Glucose	+	+	+	+	+
Sucrose	–	+	–	+	–
Maltose	–	+	–	–	–
Citrate	–	+	+	+	+
Malate	–	+	–	+	+
Adipate	–	+	–	NA	+
Succinate	–	+	+	+	+
L-Alanine	–	+	+	+	+
Nitrate respiration with carbon substrates	–	+	–	–	–†
Yeast extract or biotin requirement for optimum growth	+	–	+	+	–
Pathways/enzymes of glucose oxidation					
Embden–Meyerhof	–	+	+	NA	NA
Entner–Doudoroff	–	+	+	NA	NA
Pentose phosphate pathway	+	+	+	NA	NA
6-Phosphogluconate dehydratase	–	+	+	NA	NA
6-Phosphogluconate dehydrogenase (NAD-linked)	+	–	NA	NA	NA
6-Phosphogluconate dehydrogenase (NADP-linked)	–	+	+	NA	NA
Phosphoketolase [nmol min ^{–1} (mg protein) ^{–1}]	70 ¹	2–6 ²	NA	NA	NA
Phosphoenolpyruvate carboxylase	+ ³	+ ⁴	+ ⁵	+ ⁶	+ ⁷
Pyruvate carboxylase	+ ³	+ ⁴	– ⁵	NA	NA ⁸

NA, Data not available in the literature.

* Some strains only (Urakami *et al.*, 1995; Doronina *et al.*, 1996).

† Strain IRBG 46 reported to produce ATP during dissimilatory nitrate reduction (Raju *et al.*, 1997).

‡ Low activity [9–10 nmol NADP⁺ reduced min^{–1} (mg protein)^{–1}] (Raj, 1977).

References: 1, Greenley & Smith (1979); 2, Wood & Kelly (1980); 3, Charles & Willer (1984); 4, Smith *et al.* (1980); 5, Mullerkraft *et al.* (1991); 6, Wiegel, 1991; 7, Dunn (1998); 8, pyruvate carboxylase is produced by *Rhizobium* (Dunn, 1998).

relatedness to *T. novellus* than to *Paracoccus* (Fig. 1; Rainey & Wiegel, 1996; Stubner *et al.*, 1998). Of these, *Rhodoplanes* is a phototroph (Hiraishi & Ueda, 1994) and is only distantly related. *Azorhizobium* and *Xanthobacter* share some physiological similarities with *T. novellus* (Table 1; Dreyfus *et al.*, 1988; Padden *et al.*,

1997), but are clearly distinct from it at the genus level. *Ancylobacter aquaticus* (Ørskov, 1928; Raj, 1977, 1983, 1989) appears to be its closest phylogenetic relative (Fig. 1) and shares some physiological similarities with *T. novellus*, including the ability to grow autotrophically with formate, thiosulfate or tetrathio-

nate (Table 1); apparently, unlike *T. novellus*, it also grows autotrophically on methanol (Raj, 1977, 1989). *A. aquaticus* cells, however, contain gas vacuoles and are sharply curved or vibrioid rods, sometimes forming circles (Raj, 1977). *A. aquaticus* also exhibits a multiple-pathway mechanism of glucose oxidation similar to that of *P. versutus* (Raj, 1989) and differs in some other nutritional features from *T. novellus* (Table 1). For example, the anaplerotic carbon dioxide-fixing enzyme phosphoenolpyruvate carboxylase is produced during growth on glucose by both *T. novellus* and *A. aquaticus*, but while comparable or higher activities of pyruvate carboxylase are found in *T. novellus*, that enzyme is absent from all *Ancylobacter* strains tested, whether grown on glucose or methanol (Charles & Willer, 1984; Mullerkrantz *et al.*, 1991). This diversity of distinctive properties is inconsistent with a close generic relationship between *Ancylobacter* and *T. novellus* (Table 1), supporting the conclusion that *T. novellus* represents a distinct genus within the α -2 subclass of the *Proteobacteria*.

Description of *Starkeya* (ex *Starkey* 1935a) gen. nov.

Starkeya (Star.ke'ya. M.L. n. *Starkeya* of Starkey, referring to Robert L. Starkey, who made important contributions to the study of soil microbiology and sulfur biochemistry).

Short rods, coccoidal or ellipsoidal cells 0.4–0.8 μm $\times$ 0.8–2.0 μm , occurring singly and, occasionally, in pairs. Non-motile. Colonies grown on thiosulfate agar (with biotin) are small, smooth, circular, round and opalescent, becoming white with sulfur. Thiosulfate liquid medium (lacking biotin) becomes turbid and sulfur precipitates during static incubation: thiosulfate is incompletely used; and the pH falls from 7.8 to 5.8. This poor development is due to the requirement for biotin exhibited by the type strain. The organism is facultatively chemolithoautotrophic, but optimal autotrophic development requires biotin, and optimal heterotrophic growth requires yeast extract or biotin or other additions such as pantothenate, depending on the organic substrate. Autotrophic growth is also observed with formate, when high levels of ribulose 1,5-bisphosphate carboxylase are expressed. Some strains may degrade methylated sulfides. This organism is strictly aerobic, both autotrophically and heterotrophically, and is incapable of denitrification. Oxidizes and grows on thiosulfate and tetrathionate but not on sulfur or thiocyanate. Ammonium salts, nitrates, urea and glutamate are used as nitrogen sources. The optimum temperature is 25–30 $^{\circ}\text{C}$; the temperature for growth is in the range 10–37 $^{\circ}\text{C}$ (with no growth occurring at 5 or 42 $^{\circ}\text{C}$). The optimum pH is 7.0; the pH for growth is in the range 5.7–9.0. Contains ubiquinone Q-10. Major cellular fatty acids are octadecenoic acid and cyclopropane acid of C_{19} ; lacks a major hydroxy-fatty acid. LPS lacks heptoses and has only 2,3-diamino-2,3-dideoxyglucose as the backbone sugar. The G + C content of the DNA is 67.3–68.4 mol % (Bd, T_m). Member of the α -2 subclass

of the *Proteobacteria*. Isolated from soil and presumably widely distributed.

Description of *Starkeya novella* (*Thiobacillus novellus* Starkey 1935, 197^{Al}) gen. nov., comb. nov.

Starkeya novella (no.vel'la. L. dim. adj. *novella* new).

The species description is the same as the genus description, with the exception that oxidation of methylated sulfides has not been tested with the type strain. Biotin is required by the type strain for good growth on most substrates; yeast extract may be substituted for biotin and in some cases the biotin requirement may be replaced by lipoic acid or co-enzyme A; good growth on methanol requires pantothenate or yeast extract rather than biotin. The type strain is ATCC 8093^T (= NCIMB 10456^T = NCIMB 9113^T = DSM 506^T = IAM 12100^T = IFO 12443^T = CCM 1077^T).

Acknowledgements

We thank Erko Stackebrandt and Hans Trüper for advice and encouragement.

References

- Beffa, T., Fisher, C. & Aragno, M. (1993). Growth and respiratory oxidation of reduced sulfur compounds by intact cells of *Thiobacillus novellus* (type strain) grown on thiosulfate, *Curr Microbiol* **26**, 323–326.
- Cha, J. M., Cha, W. S. & Lee, J. H. (1999). Removal of organo-sulphur odour compounds by *Thiobacillus novellus* SRM, sulphur-oxidizing microorganisms, *Process Biochem* **34**, 659–665.
- Chandra, T. S. & Shethna, Y. I. (1977). Oxalate, formate, formamide, and methanol metabolism in *Thiobacillus novellus*, *J Bacteriol* **131**, 389–398.
- Charles, A. M. & Suzuki, I. (1966). Mechanism of thiosulfate oxidation by *Thiobacillus novellus*, *Biochim Biophys Acta* **128**, 510–521.
- Charles, A. M. & Willer, D. W. (1984). Pyruvate carboxylase from *Thiobacillus novellus*: properties and possible function, *Can J Microbiol* **30**, 532–539.
- Doronina, N. V., Trotsenko, Y. A., Krautzova, V. I. & Suzina, N. E. (1996). New methylotrophic isolates of the genus *Xanthobacter*, *Microbiology* (English translation of *Mikrobiologiya*) **65**, 217–224.
- Dreyfus, B., Garcia, J. L. & Gillis, M. (1988). Characterization of *Azorhizobium caulinodans* gen. nov., sp. nov., a stem-nodulating nitrogen-fixing bacterium isolated from *Sesbania rostrata*, *Int J Syst Bacteriol* **38**, 89–98.
- Dunn, M. F. (1998). Tricarboxylic acid cycle and anaplerotic enzymes in rhizobia, *FEMS Microbiol Rev* **22**, 105–123.
- Feldmann, K. & Goroll, D. (1976). Abbau von Schwefelverbindungen durch *Thiobacillus*-Reinkulturen, *Z Allg Mikrobiol* **16**, 287–290.
- Greenley, D. E. & Smith, D. W. (1979). A novel pathway of glucose catabolism in *Thiobacillus novellus*, *Arch Microbiol* **122**, 257–261.

- Hamana, K. & Matsuzaki, S. (1990). Five types of polyamine distribution patterns in thiobacilli, *FEMS Microbiol Lett* **70**, 347–352.
- Harrison, A. P. (1983). Genomic and physiological comparisons between heterotrophic thiobacilli and *Acidiphilium cryptum*, *Thiobacillus versutus* sp. nov., and *Thiobacillus acidophilus* nom. rev, *Int J Syst Bacteriol* **33**, 211–217.
- Hiraishi, A. & Ueda, Y. (1994). *Rhodoplanes* gen. nov., a new genus of phototrophic bacteria including *Rhodopseudomonas rosea* as *Rhodoplanes roseus* comb. nov. and *Rhodoplanes elegans* sp. nov, *Int J Syst Bacteriol* **44**, 665–673.
- Hiraishi, A., Furuhashi, K., Matsumoto, A., Koike, K. A., Fukuyama, M. & Tabuchi, K. (1995). Phenotypic and genetic diversity of chlorine-resistant *Methylobacterium* strains isolated from various environments, *Appl Environ Microbiol* **61**, 2099–2107.
- Hiraishi, A., Nagashima, K. V. P. & Katayama, Y. (1998). Phylogeny and photosynthetic features of *Thiobacillus acidophilus* and related acidophilic bacteria: its transfer to the genus *Acidiphilium* as *Acidiphilium acidophilum* comb. nov, *Int J Syst Bacteriol* **48**, 1389–1398.
- Holmes, A. J., Kelly, D. P., Baker, S. C., Thompson, A. S., De Marco, P., Kenna, E. M. & Murrell, J. C. (1997). *Methylosulfonomonas methylovora* gen. nov., sp. nov., and *Marinosulfonomonas methylophila* gen. nov., sp. nov.: novel methylophils able to grow on methanesulfonic acid, *Arch Microbiol* **167**, 46–53.
- Hutchinson, M., Johnstone, K. I. & White, D. (1965). The taxonomy of certain thiobacilli, *J Gen Microbiol* **41**, 357–366.
- Hutchinson, M., Johnstone, K. I. & White, D. (1969). Taxonomy of the genus *Thiobacillus*: the outcome of numerical taxonomy applied to the group as a whole, *J Gen Microbiol* **57**, 397–410.
- Katayama, Y., Hiraishi, A. & Kuraishi, H. (1995). *Paracoccus thiocyanatus* sp. nov., a new species of thiocyanate-utilizing facultative chemolithotroph, and transfer of *Thiobacillus versutus* to the genus *Paracoccus* as *Paracoccus versutus* comb. nov. with emendation of the genus, *Microbiology* **141**, 1469–1477.
- Katayama-Fujimura, Y. & Kuraishi, H. (1980). Characterization of *Thiobacillus novellus* and its thiosulfate oxidation, *J Gen Appl Microbiol* **26**, 357–367.
- Kelly, D. P. (1989). Physiology and biochemistry of unicellular sulfur bacteria. In *Autotrophic Bacteria*, pp. 193–217. Edited by H. G. Schlegel & B. Bowien. Madison/Berlin: Science Tech Publishers/Springer.
- 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. (1982). Autotrophic growth of *Thiobacillus* A2 on methanol, *FEMS Microbiol Lett* **15**, 229–233.
- Kelly, D. P. & Wood, A. P. (2000a). Reclassification of some species of *Thiobacillus* to the newly designated genera, *Acidithiobacillus* gen. nov., *Halothiobacillus* gen. nov. and *Thermithiobacillus* gen. nov, *Int J Syst Evol Microbiol* **50**, 511–516.
- Kelly, D. P. & Wood, A. P. (2000b). Confirmation of *Thiobacillus denitrificans* as a species of the genus *Thiobacillus*, in the β -subclass of the *Proteobacteria*, with strain NCIMB 9548 as the type strain, *Int J Syst Evol Microbiol* **50**, 547–550.
- Kelly, D. P., Wood, A. P., Gottschal, J. C. & Kuenen, J. G. (1979). Autotrophic growth on formate by *Thiobacillus* strain A2, *J Gen Microbiol* **114**, 1–13.
- Kelly, D. P., Shergill, J. K., Lu, W.-P. & Wood, A. P. (1997). Oxidative metabolism of inorganic sulfur compounds by bacteria, *Antonie Leeuwenhoek* **71**, 95–107.
- Kelly, D. P., Wood, A. P. & Stackebrandt, E. (2000). Genus *Thiobacillus* Beijerinck. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2 (in press). Edited by N. R. Krieg, J. T. Staley & D. J. Brenner. Michigan: Bergey's Manual Trust.
- Lane, D. J., Stahl, D. A., Olsen, G. J., Heller, D. J. & Pace, N. R. (1985). Phylogenetic analysis of the genera *Thiobacillus* and *Thiomicrospira* by 5S rRNA sequences, *J Bacteriol* **163**, 75–81.
- Lu, W.-P. & Kelly, D. P. (1983). Partial purification and resolution of a thiosulphate-oxidizing system from *Thiobacillus* A2, *J Gen Microbiol* **129**, 1673–1681.
- Lu, W.-P., Swoboda, B. E. P. & Kelly, D. P. (1985). Properties of the thiosulphate-oxidizing multi-enzyme system from *Thiobacillus versutus*, *Biochim Biophys Acta* **828**, 116–122.
- McDonald, I. R., Kelly, D. P., Murrell, J. C. & Wood, A. P. (1997). Taxonomic relationships of *Thiobacillus halophilus*, *Thiobacillus aquaesulii*, and other species of *Thiobacillus*, as determined using 16S rRNA sequencing, *Arch Microbiol* **166**, 394–398.
- Matin, A. & Rittenberg, S. C. (1971). Enzymes of carbohydrate metabolism in *Thiobacillus* species, *J Bacteriol* **107**, 179–186.
- 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.
- Mullerkrantz, G., Uhlenhuth, J. & Babel, W. (1991). Heterotrophic carbon dioxide fixation by a methylotrophic *Ancylobacter* species, *J Basic Microbiol* **31**, 317–319.
- Oh, J. K. & Suzuki, I. (1977a). Isolation and characterization of a membrane-associated thiosulphate-oxidizing system of *Thiobacillus novellus*, *J Gen Microbiol* **99**, 397–412.
- Oh, J. K. & Suzuki, I. (1977b). Resolution of a membrane-associated thiosulphate-oxidizing system of *Thiobacillus novellus*, *J Gen Microbiol* **99**, 413–423.
- Ørskov, J. (1928). Beschreibung eines neuen Mikroben, *Microcycylus aquaticus*, mit eigentümlicher Morphologie, *Zentbl Bakteriell Parasitenkd Abt I* **107**, 180–184.
- Padden, A. N., Rainey, F. A., Kelly, D. P. & Wood, A. P. (1997). *Xanthobacter tagetidis* sp. nov., an organism associated with *Tagetes* species and able to grow on substituted thiophenes, *Int J Syst Bacteriol* **47**, 394–401.
- Rainey, F. A. & Wiegand, J. (1996). 16S ribosomal DNA sequence analysis confirms the close relationship between the genera *Xanthobacter*, *Azorhizobium* and *Aquabacter* and reveals a lack of phylogenetic coherence among *Xanthobacter* species, *Int J Syst Bacteriol* **46**, 607–610.
- Rainey, F. A., Kelly, D. P., Stackebrandt, E., Burghardt, J., Hiraishi, A., Katayama, Y. & Wood, A. P. (1999). A re-evaluation of the taxonomy of *Paracoccus denitrificans* and a proposal for the combination *Paracoccus pantotrophus* comb. nov, *Int J Syst Bacteriol* **49**, 645–651.
- Raj, H. D. (1977). *Microcycylus* and related ring-forming bacteria, *CRC Crit Rev Microbiol* **5**, 243–270.
- Raj, H. D. (1983). Proposal for *Ancylobacter* gen. nov. as a substitute for the bacterial genus *Microcycylus* Ørskov 1928, *Int J Syst Bacteriol* **33**, 397–398.
- Raj, H. D. (1989). Oligotrophic methylotrophs – *Ancylobacter* (basonym “*Microcycylus*” Ørskov) Raj gen. nov, *CRC Crit Rev Microbiol* **17**, 89–106.
- Raju, K. S., Sharma, N. D. & Lodha, M. L. (1997). ATP production during dissimilatory nitrate reduction in *Azorhizobium caulinodans* IRBG 46, *FEMS Microbiol Lett* **151**, 17–21.

- Sijderius, R. (1946).** *Heterotrophe bacteriën, die thiosulfaat oxydeeren*. Doctoral thesis, Universiteit van Amsterdam. Deventer: Kluwer.
- Smith, A. L., Kelly, D. P. & Wood, A. P. (1980).** Metabolism of *Thiobacillus* A2 grown under autotrophic, mixotrophic and heterotrophic conditions in chemostat culture, *J Gen Microbiol* **121**, 127–138.
- Starkey, R. L. (1934).** Cultivation of organisms concerned in the oxidation of thiosulfate, *J Bacteriol* **28**, 365–386.
- Starkey, R. L. (1935a).** Isolation of some bacteria which oxidize thiosulfate, *Soil Sci* **39**, 197–219.
- Starkey, R. L. (1935b).** Products of the oxidation of thiosulfate by bacteria in mineral media, *J Gen Physiol* **18**, 325–349.
- Stubner, S., Wind, T. & Conrad, R. (1998).** Sulfur oxidation in rice field soil: activity, enumeration, isolation and characterization of thiosulfate-oxidizing bacteria, *Syst Appl Microbiol* **21**, 569–578.
- Taylor, B. F. & Hoare, D. S. (1969).** New facultative *Thiobacillus* and a re-evaluation of the heterotrophic potential of *Thiobacillus novellus*, *J Bacteriol* **100**, 487–497.
- Urakami, T., Araki, H. & Komagata, K. (1995).** Characteristics of newly isolated *Xanthobacter* strains and fatty acid compositions and quinone systems in yellow-pigmented hydrogen-oxidizing bacteria, *Int J Syst Bacteriol* **45**, 863–867.
- Vishniac, W. & Santer, M. (1957).** The thiobacilli, *Bacteriol Rev* **21**, 195–213.
- Wiegel, J. (1991).** The genus *Xanthobacter*. In *The Prokaryotes*, 2nd edn, vol. 3, pp. 2365–2383. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. New York: Springer.
- Wood, A. P. & Kelly, D. P. (1977).** Heterotrophic growth of *Thiobacillus* A2 on sugars and organic acids, *Arch Microbiol* **113**, 257–264.
- Wood, A. P. & Kelly, D. P. (1978).** Triple catabolic pathways for glucose in a fast-growing strain of *Thiobacillus* A2, *Arch Microbiol* **117**, 309–310.
- Wood, A. P. & Kelly, D. P. (1980).** Carbohydrate degradation pathways in *Thiobacillus* A2 grown in various sugars, *Arch Microbiol* **120**, 333–345.
- Wood, A. P., Kelly, D. P. & Thurston, C. F. (1977).** Simultaneous operation of three catabolic pathways in the metabolism of glucose by *Thiobacillus* A2, *Arch Microbiol* **113**, 265–274.