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NOTE

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

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***Thiobacillus denitrificans* is physiologically similar to the type species of the genus *Thiobacillus*, *Thiobacillus thioparus*, and both are located in the β-subclass of the *Proteobacteria*. *T. denitrificans* is distinguished from all other *Thiobacillus* species by its ability to grow as a facultatively anaerobic chemolithotroph, coupling the oxidation of inorganic sulfur compounds to the reduction of nitrate, nitrite and other oxidized nitrogen compounds to dinitrogen. A definitive description of this species is provided and strain NCIMB 9548^T is designated as the type strain of the species, thereby correcting an earlier error in the literature.**

Keywords: *Thiobacillus denitrificans*, type strain NCIMB 9548^T, *Thiobacillus* taxonomy, 16S rDNA phylogeny, β-Proteobacteria

At the time of the publication of the first edition of *Bergey's Manual of Systematic Bacteriology* (Kelly & Harrison, 1989a), *Thiobacillus denitrificans* was not on the approved lists of bacterial names (Skerman *et al.*, 1980). The original isolate (Beijerinck, 1904a, b) may not have been a pure culture (Vishniac & Santer, 1957); that culture is no longer available in the Delft Culture Collection (L. A. Robertson, personal communication) or in any other culture collection. Later work proved that Beijerinck's (1904a) designation was of a valid species exhibiting stable physiological characteristics (Lieske, 1912; Baalsrud & Baalsrud, 1954; Hutchinson *et al.*, 1967; Taylor *et al.*, 1971; Baldensberger & Garcia, 1975; Justin & Kelly, 1978a; Katayama-Fujimura *et al.*, 1982). Reports that its capacity to denitrify was lost upon aerobic subculture and that it was facultatively heterotrophic were erroneous. The name *T. denitrificans* was therefore revived (Kelly & Harrison, 1989a) with a newly suggested neotype or reference strain, since the early isolates were no longer extant. The name appeared in *International Journal of Systematic Bacteriology* Validation List no. 31 (Kelly & Harrison, 1989b), but unfortunately the culture collection strain designated as representative of the type strain was incorrectly given as NCIB 8327. The correct designation is NCIMB 9548^T; this correction was published by the National Collection of Industrial and Marine Bacteria (NCIMB, 1994) and was corrected for the second edition of *Bergey's Manual of Systematic Bacteriology* (Kelly & Wood, 2000a).

This paper assesses the designation of *T. denitrificans* as a valid species of *Thiobacillus* and provides a definitive description of the species, with a clarification of the availability of the type strain in culture collections.

Status of the genus *Thiobacillus* Beijerinck (1904a)

The currently recognized species of *Thiobacillus* exhibit an extraordinarily wide range of physical growth conditions and great diversity in terms of the G+C content of their DNA (50–68 mol%), the extent of DNA hybridization, the range of ubiquinones and fatty acid content (Kelly & Harrison, 1989a; Kelly & Wood, 2000a). This diversity of properties among species was indicative of an extremely heterogeneous group, judged in terms of genetic and physiological similarity, and 16S rDNA sequence analysis confirmed extreme diversity and consequent misclassification among the thiobacilli (McDonald *et al.*, 1997). Consequently, various workers have proposed the assignment of some species to other genera or the creation of

The EMBL accession number for the 16S rRNA sequence of *Thiobacillus denitrifican* is AJ243144.

new genera for most of the former species of *Thiobacillus* (Katayama *et al*., 1995; Moreira & Amils, 1997; Hiraishi *et al*., 1998; Kelly & Wood, 2000b). In writing the chapter on *Thiobacillus* for the second edition of *Bergey's Manual of Systematic Bacteriology*, we have confirmed the assignment of 14 species to new or different genera (Kelly & Wood, 2000a, b). Of the remaining *Thiobacillus* species, only *T. denitrificans* is capable of facultative anaerobic growth with denitrification.

The status of *T. denitrificans* after revision of the genus *Thiobacillus* in 1999

The type species of the *Thiobacillus* genus, *Thiobacillus thioparus* (Beijerinck, 1904a), is a member of the β-subclass of the *Proteobacteria*, as is *T. denitrificans* (Woese *et al*., 1984; Lane *et al*., 1992; H. G. Trüper, personal communication). *T. denitrificans*, like *T. thioparus*, exhibits obligate chemolithoautotrophy on a range of inorganic sulfur compounds and has numerous other physiological similarities in terms of, for example, pH- and temperature optima, the possession of ubiquinone Q-8, DNA base composition and fatty acid profiles (Agate & Vishniac, 1973; Katayama-Fujimura *et al*., 1982). *T. denitrificans* is primarily characterized by its ability to grow as a facultative anaerobe, using nitrate and other oxidized nitrogen compounds in place of oxygen, a capacity that is absent in *T. thioparus*. A major structural difference between *T. thioparus* and *T. denitrificans* is shown by the presence (*T. thioparus*) or absence (*T. denitrificans*) of carboxysomes (polyhedral bodies largely composed of ribulose bisphosphate carboxylase) (Katayama-Fujimura *et al*., 1984; Shively *et al*., 1970). The wide range of comparative tests employed by Hutchinson *et al*. (1967, 1969) confirmed many similarities between *T. denitrificans* and *T. thioparus*, but showed them to be different from each other at the species level (Hutchinson *et al*., 1967). Recently, the 16S rRNA gene sequence of *T. denitrificans* NCIMB 9548^T was shown to have 98% similarity to that of the type strain of *T. thioparus* (ATCC 8185^T; E. Stackebrandt, personal communication). The two species showed only 22–29% DNA–DNA hybridization (Katayama-Fujimura *et al*., 1983), confirming them as distinct species.

A striking feature of *T. denitrificans* is its greater growth yields on thiosulfate, both aerobically and anaerobically, relative to those of *T. thioparus*. Aerobic yields on thiosulfate or tetrathionate are approximately double those of *T. thioparus* and other physiologically similar chemolithotrophs (Justin & Kelly, 1978a, b; Kelly, 1990, 2000a; Timmer-ten Hoor, 1976, 1981). One characteristic of *T. thioparus* is its ability to grow on thiocyanate as a sole source of energy (Katayama *et al*., 1992; Kelly & Harrison, 1989a), a property apparently shared only with *T. denitrificans* (among the thiobacilli). Beijerinck (1904a) reported 0·25% ammonium thiocyanate giving rise to a profuse deposition of elemental sulfur when provided in place of thiosulfate in the culture medium. Thiocyanate consumption was reported by Hutchinson *et al*. (1965, 1967, 1969) as a taxonomic characteristic of both *T. denitrificans* and *T. thioparus*. No quantitative study of growth coupled to thiocyanate breakdown by *T. denitrificans* appears to have been done. De Kruyff *et al*. (1957) and Van der Walt & De Kruyff (1955) showed both aerobic and nitrate-dependent anaerobic use of thiocyanate by a strain of *T. denitrificans*: thiocyanate (2·6 mM) was quantitatively converted to sulfate (87–94%) and elemental sulfur (6–13%). Thus, a definitive study of the biochemistry of thiocyanate metabolism in *T. denitrificans* is still required. Beijerinck's (1904a) hope of returning to this question ('… ein eigentümlicher Biochemismus obwaltet, auf den ich später zurückzukommen hoffe') seems to have been unfulfilled. Lieske (1912) reported growth ('ein sehr gutes Wachstum') of *T. denitrificans* on sodium dithionate ($Na_2S_2O_6$) but this was not confirmed by Hutchinson *et al*. (1967, 1969), although they did report weak use of dithionate by *T. thioparus* (Hutchinson *et al*., 1969). There seems to have been no other report of dithionate as a sole substrate by *T. denitrificans*; quantitative studies of both thiocyanate and dithionate as possible substrates remain to be performed with this organism.

Description of *Thiobacillus denitrificans* (*ex* Beijerinck 1904a) nom. rev. Kelly and Harrison (1989a)

Thiobacillus denitrificans (de.ni.tri′fi.cans. M.L. v. *denitrifico* denitrify; M.L. part. adj. *denitrificans* denitrifying).

Short rods 0·5 × 1·0–3·0 μm in size. May be motile by means of a polar flagellum. Clear or weakly opalescent colonies are grown anaerobically on thiosulfate/nitrate agar, which upon ageing may become white with sulfur. Growth in anaerobic stab- or roll culture results in agar splitting due to production of nitrogen gas. Facultatively anaerobic. Grows as an aerobic chemolithoautotroph on thiosulfate, tetrathionate and thiocyanate. Grows as an anaerobic chemolithoautotroph on thiosulfate, tetrathionate, thiocyanate, sulfide or elemental sulfur by using nitrate, nitrite or nitrous oxide as terminal respiratory oxidants; transient formation and consumption of nitric oxide has been observed. Oxidizes sulfur, sulfide, thiosulfate, tetrathionate, sulfite and thiocyanate. Batch cultures can be grown in completely filled bottles, producing vigorous nitrogen evolution. Chemostat culture can be switched easily and repeatedly between aerobic and anaerobic growth modes, with adaptation involving derepression of nitrate and nitrite reductase synthesis. Ammonium salts and, in some strains at least, nitrate are used as nitrogen sources. Obligately chemolithotrophic and autotrophic. Optimum temperature 28–32 °C. Optimum pH 6·8–7·4. Found in soil, mud, freshwater- and marine sediments and also in domestic sewage and industrial waste-treatment lagoons and

digestion tanks, especially under anoxic conditions. Probably very widely distributed. The G+C content of the DNA is 63 mol% (Bd, T_m). The type strain is NCIMB 9548^T. This is the strain (AB7) deposited by Hutchinson *et al.* (1967), which is also available as ATCC 23644^T and JCM 3870^T. A member of the *β-Proteobacteria*.

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