

## Reclassification of *Trichlorobacter thiogenes* as *Geobacter thiogenes* comb. nov.

Kelly P. Nevin, Dawn E. Holmes, Trevor L. Woodard, Sean F. Covalla and Derek R. Lovley

Department of Microbiology, University of Massachusetts, Amherst, MA 01003, USA

### Correspondence

Kelly P. Nevin

knevin@microbio.umass.edu

Reclassification of the species *Trichlorobacter thiogenes* as *Geobacter thiogenes* comb. nov. is proposed on the basis of physiological traits and phylogenetic position. Characteristics additional to those provided in the original description revealed that the type strain (strain K1<sup>T</sup> = ATCC BAA-34<sup>T</sup> = JCM 14045<sup>T</sup>) has the ability to use Fe(III) as an electron acceptor for acetate oxidation and has an electron donor and acceptor profile typical of a *Geobacter* species, contains abundant *c*-type cytochromes, and has a temperature optimum of 30 °C and a pH optimum near pH 7.0; traits typical of members of the genus *Geobacter*. Phylogenetic analysis of *nifD*, *recA*, *gyrB*, *rpoB*, *fusA* and 16S rRNA genes further indicated that *T. thiogenes* falls within the *Geobacter* cluster of the family *Geobacteraceae*. Based on extensive phylogenetic evidence and the fact that *T. thiogenes* has the hallmark physiological characteristics of a *Geobacter* species, *Trichlorobacter thiogenes* should be reclassified as a member of the genus *Geobacter*.

*Trichlorobacter thiogenes* strain K1<sup>T</sup> (=ATCC BAA-34<sup>T</sup> = JCM 14045<sup>T</sup>) (De Wever *et al.*, 2000) was affiliated with the genus *Trichlorobacter* mainly on the basis of its ability to dechlorinate trichloroacetic acid. However, this Gram-negative, non-motile, short curved rod has the ability to use acetate as an electron donor, similar to all *Geobacter* species in the family *Geobacteraceae* (Lovley *et al.*, 2004). *T. thiogenes* also shares other important physiological traits with many *Geobacter* species, such as S<sup>0</sup> and fumarate reduction (Lovley *et al.*, 2004). Immediately after the publication of the original description of *T. thiogenes* (De Wever *et al.*, 2000), a phylogenetic study of 16S rRNA genes from the 12 available *Geobacter* species that had been described at that time showed that the type strain of *T. thiogenes* fell within the *Geobacter* clade of the family *Geobacteraceae* (Snoeyenbos-West *et al.*, 2001). The response of De Wever *et al.* to this study stated that, at the time of publication of the description of *T. thiogenes*, only two *Geobacter* species, *Geobacter metallireducens* (ATCC 53774<sup>T</sup> = DSM 7210<sup>T</sup>) and *Geobacter sulfurreducens* (ATCC 51573<sup>T</sup> = DSM 12127<sup>T</sup>), had validly published names and that phylogenetic analysis of additional genes (*gyrB*) was necessary (De Wever *et al.*, 2001a). Subsequently, the names of other *Geobacter* species, *Geobacter bremsensis* (ATCC BAA-607<sup>T</sup> = DSM 12179<sup>T</sup> = Dfr1<sup>T</sup> = OCM 796<sup>T</sup>), *Geobacter pelophilus* (ATCC BAA-603<sup>T</sup> = DSM 12255<sup>T</sup> = Dfr2<sup>T</sup> = OCM 797<sup>T</sup>) (Straub & Buchholz-Cleven, 2001), *Geobacter chapellei* (DSM 13688<sup>T</sup>), *Geobacter grbiciae* (ATCC BAA-45<sup>T</sup>), *Geobacter hydrogenophilus* (ATCC 51590<sup>T</sup> = DSM 13691<sup>T</sup>) (Coates *et al.*, 2001), *Geobacter psychrophilus* (JCM 12644<sup>T</sup>) and *Geobacter bemidjiensis*

(ATCC BAA-1014<sup>T</sup>) (Nevin *et al.*, 2005), have been validly published. Furthermore, a phylogenetic study of the *nifD*, *recA*, *gyrB*, *rpoB*, *fusA* and 16S rRNA genes from 30 members of the *Geobacteraceae* has been conducted and the sequences deposited in GenBank/EMBL/DDBJ. The nucleotide and amino acid sequences of the following genes have been compared: *rpoB*, encoding the  $\beta$ -subunit of RNA polymerase; *recA*, encoding the DNA repair protein, RecA; *gyrB*, the structural gene for the DNA gyrase  $\beta$ -subunit; *fusA*, encoding the protein synthesis elongation factor, elongation factor-G; *nifD*, encoding the  $\alpha$ -subunit of the dinitrogenase protein; and the 16S rRNA gene. The results demonstrate that the *Geobacteraceae* is a phylogenetically and physiologically distinct family within the *Deltaproteobacteria* and that *T. thiogenes* is clearly a member of the *Geobacter* clade of this family (Holmes *et al.*, 2004).

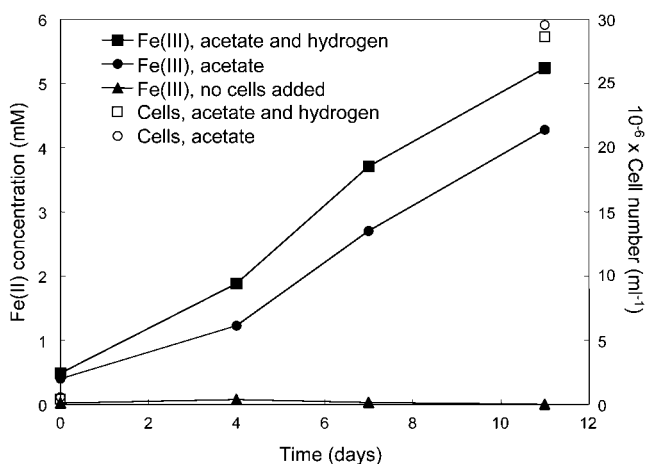
Cytochrome analysis was performed on *T. thiogenes*, *G. chapellei* and *G. sulfurreducens* using cells grown in media described in the original descriptions with fumarate as electron acceptor and acetate as electron donor (Lovley *et al.*, 2004). Three millilitres of culture was resuspended in 20 mM PIPES (pH 7) and spectra were obtained as described previously (Caccavo *et al.*, 1994) using a Shimadzu UV2401-PC dual beam spectrophotometer. The dithionite-reduced minus air-oxidized difference spectrum of *T. thiogenes* and *G. chapellei* showed the presence of *c*-type cytochromes, with absorbance peaks at 420 and 552 nm and a shoulder at 522 nm. A similar spectrum was obtained from the control, *G. sulfurreducens*, with peaks and a shoulder at the same absorbance values. The presence of *c*-type

cytochromes is a distinguishing feature of the family *Geobacteraceae*, with the exception of members of the genus *Pelobacter*. The cells of *T. thiogenes* are visibly pink, as are the cells of all *Geobacter* species.

*T. thiogenes* has not been reported previously to be able to reduce Fe(III). Fe(III) reduction is considered to be a hallmark trait of species of the genus *Geobacter*. Therefore, the Fe(III) reduction capability of *T. thiogenes* was evaluated. Cells of *T. thiogenes* were transferred four times in basal media (De Wever *et al.*, 2000) containing 5 mM Fe(III) nitritoacetate, 5 mM acetate and 0.01 g yeast extract  $l^{-1}$ , in the presence and absence of hydrogen. Medium from the fifth transfer was monitored for Fe(II) using the ferrozine method (Lovley & Phillips, 1986) to evaluate Fe(III) reduction, and direct cell counts (Lovley & Phillips, 1988) were carried out to determine cell growth. *T. thiogenes* conserved energy for growth from the oxidation of acetate coupled to the reduction of Fe(III) (Fig. 1). The addition of hydrogen slightly increased the amount of Fe(III) reduced, but had no effect on cell number. The reduction of Fe(III) is a distinguishing characteristic of the family *Geobacteraceae*. All *Geobacter* species are capable of Fe(III) reduction, with acetate serving as the electron donor (Lovley *et al.*, 2004).

*T. thiogenes* was tested for its ability to use a variety of electron donors and acceptors that its closest relative, *G. chapellei*, was capable of utilizing. A comparison between the electron donor and acceptor profiles of these two organisms is given in Table 1, together with DNA–DNA hybridization values, DNA G+C content, temperature optimum and range and cytochrome content.

The temperature and pH range and optima for growth of *T. thiogenes* were determined. Tubes containing medium with fumarate as electron acceptor and acetate as electron donor, in triplicate, were used to determine growth at 4, 10, 17, 22,



**Fig. 1.** Growth of cells of *G. thiogenes* strain K1<sup>T</sup> in basal medium with Fe(III) nitritoacetate as electron acceptor and acetate or acetate plus hydrogen as electron donor.

30, 37 and 42 °C. Growth was evaluated using OD<sub>600</sub> values and microscopic examination. Growth occurred at 10, 17, 22, 30 and 37 °C, with optimum growth at 30 °C, which is typical of many *Geobacter* species. A circum-neutral pH optimum for growth was observed in media containing fumarate as electron acceptor and acetate as electron donor using OD<sub>600</sub> values and microscopic examination.

Previous studies have suggested that placement of *T. thiogenes* in the appropriate genus in the *Geobacteraceae* should be based on phylogenetic comparisons of genes other than the 16S rRNA gene alone (De Wever *et al.*, 2000,

**Table 1.** Physiological characteristics of *Geobacter thiogenes* strain K1<sup>T</sup> and *Geobacter chapellei* strain 172<sup>T</sup> including use of various electron donors with fumarate as electron acceptor and various electron acceptors with acetate as electron donor

Unless indicated otherwise data for strain K1<sup>T</sup> were taken from Jurgen Wiegel and Frank Löffler, this study and De Wever *et al.* (2000). Data for strain 172<sup>T</sup> were taken from Coates *et al.* (2001) and this study. Acetate (10 mM) and 2,6-anthraquinone disulfonate (AQDS; 5 mM) tested positive as electron donors for the two strains; benzoate (2 mM), butyrate (10 mM), malate (10 mM), propionate (10 mM), pyruvate (10 mM) and succinate (10 mM) tested negative as electron donors for the two strains. Fe(III) pyrophosphate (10 mM), Fe(III) nitritoacetate (5 mM) and fumarate (10–40 mM) tested positive as electron acceptors for the two strains; Fe(III) citrate (55 mM), sulfate (10 mM) and thiosulfate (10 mM) tested negative as electron acceptors for the two strains. The DNA–DNA hybridization value between strains K1<sup>T</sup> and 172<sup>T</sup> is 13% (data from DSMZ) and both strains contain cytochromes. NT, Not tested.

Characteristic	<i>Geobacter thiogenes</i> strain K1 <sup>T</sup>	<i>Geobacter chapellei</i> strain 172 <sup>T</sup>
DNA G+C content (mol%)	55.1	50.2
Temperature optimum (°C)	30	25
Temperature range (°C)	10–37	NT
Donors:		
Acetoin (10 mM)	+	–
Ethanol (10 mM)	–	+
Formate (10 mM)	–	+
Hydrogen (130 kPa)*	+	–
Lactate (10 mM)	–	+
Acceptors:		
Elemental sulfur (20 g $l^{-1}$ )	+	–
Fe(III) oxide (100 mM)	NT	+
Nitrate (5 mM)	+	–
Malate (10 mM)	+	–
Mn(IV) oxide (20 g $l^{-1}$ )	–	+
Trichloroacetic acid (5 mM)	+	–

\*Acetate (0.1 mM) was provided as carbon source for growth on hydrogen.

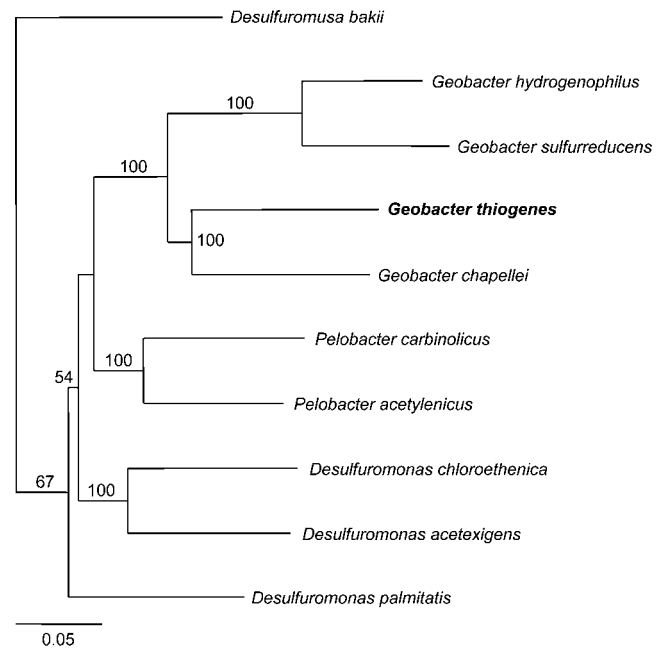
2001a). Therefore, additional phylogenetic analysis of members of the family *Geobacteraceae* was conducted using the following genes: *rpoB*, *recA*, *gyrB*, *fusA*, *nifD* and 16S rRNA (Holmes *et al.*, 2004). These additional taxonomic comparisons demonstrated that *T. thioenes* consistently groups within the freshwater *Geobacter* clade of the *Geobacteraceae* (Holmes *et al.*, 2004). Similarity matrices generated using the similarity matrix program (Maidak *et al.*, 2001), available on the Ribosomal database Project II website, and LFASTA version 3.2 (Pearson, 1990) demonstrated that *T. thioenes* is most similar to *G. chapellei*. The 16S rRNA gene sequence similarity between *T. thioenes* and *G. chapellei* was 93.9%, and the nucleotide and amino acid sequence similarities for the *fusA*, *gyrB*, *nifD*, *recA* and *rpoB* genes were 77.9–91.8%.

Concatamers were assembled with 1323 nucleotides from the 16S rRNA gene, 883 nucleotides from *gyrB*, 412 nucleotides from *recA*, 590 nucleotides from *fusA*, 540 nucleotides from *rpoB* and 440 nucleotides from *nifD*. The genes used to construct the concatamers were submitted separately to GenBank (Holmes *et al.*, 2004). Once constructed, the concatamers were aligned using CLUSTAL\_X (Thompson *et al.*, 1997) and imported into the Genetic Computer Group (GCG) sequence editor (Wisconsin Package version 10) where alignments were checked and hypervariable regions were masked. Aligned sequences were then imported into PAUP 4.0b 4a (Swofford, 1998), where phylogenetic distances were inferred. Comparisons of these concatamated alignments clearly demonstrate that *T. thioenes* falls within the phylogenetically coherent *Geobacter* cluster of the family *Geobacteraceae* (Fig. 2). Analysis of concatamated alignments indicated that, similar to previous gene comparisons (Holmes *et al.*, 2004), *T. thioenes* is most similar to *G. chapellei* (81.3% genetic sequence similarity).

Members of the family *Geobacteraceae* have been detected or isolated from a wide variety of aquatic sediments and subsurface sediments. The ability of *T. thioenes* to grow via reductive dehalogenation was a novel characteristic for members of the *Geobacter* cluster of the *Geobacteraceae*, but the capacity for dehalogenation has been observed previously in members of the *Desulfuromonas* cluster of this family (Krumholz *et al.*, 1996; Krumholz, 1997; Löffler *et al.*, 2000) and thus this characteristic alone does not warrant a new genus designation. Furthermore, a recently isolated strain of *Geobacter*, '*Geobacter lovleyi*' strain SZ, is also capable of dechlorination (Sung *et al.*, 2006). The finding that, like all *Geobacter* species, *T. thioenes* is capable of Fe(III) reduction and contains abundant *c*-type cytochromes and that, using six separate genes, its phylogenetic placement within the *Geobacter* clade of the *Geobacteraceae* is maintained, indicates that *T. thioenes* should be reclassified as a species of *Geobacter*.

### Description of *Geobacter thioenes* comb. nov.

*Geobacter thioenes* (thi.o'ge.nes. Gr. n. *thion* sulfur; Gr. v. *gennaos* produce; N.L. part. adj. *thioenes* producing sulfur).



**Fig. 2.** Phylogenetic tree constructed with the HKY85 distance-based algorithm showing the relationship between concatamers assembled from 16S rRNA, *recA*, *nifD*, *rpoB*, *gyrB* and *fusA* gene fragments from the type strains of *G. thioenes* and other species within the family *Geobacteraceae*. *Desulfuromusa bakii* was used as an outgroup. Bootstrap values were determined from 100 replicates. Bar, 0.05 nucleotide substitutions per site.

Basonym: *Trichlorobacter thioenes* De Wever *et al.* 2001b.

Physiological data and phylogenetic analysis based on *nifD*, *recA*, *gyrB*, *rpoB*, *fusA* and 16S rRNA genes indicate that *Trichlorobacter thioenes* is a member of the *Geobacter* clade of the family *Geobacteraceae*. Has the following properties in addition to those given in the original description. Capable of Fe(III) reduction. Cells contain abundant *c*-type cytochromes.

The type strain is K1<sup>T</sup> (= ATCC BAA-34<sup>T</sup> = JCM 14045<sup>T</sup>), which was enriched from subsoil from western Michigan with acetate as an electron donor and trichloroacetic acid as an electron acceptor.

### Acknowledgements

This research was supported by the Office of Science (BER), US Department of Energy, Cooperative Agreement no. DE-FC02-02ER63446 and Grant no. DE-FG02-97ER62475.

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