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

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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\(^T\) = ATCC BAA-34\(^T\) = JCM 14045\(^T\)) 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\(^T\) (= ATCC BAA-34\(^T\) = JCM 14045\(^T\)) (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*\(^0\) 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\(^T\) = DSM 7210\(^T\)) and *Geobacter sulfurreducens* (ATCC 51573\(^T\) = DSM 12127\(^T\)), 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 brementis* (ATCC BAA-607\(^T\) = DSM 12179\(^T\) = Dfr1\(^T\) = OCM 796\(^T\)), *Geobacter pelophilus* (ATCC BAA-603\(^T\) = DSM 12255\(^T\) = Dfr2\(^T\) = OCM 797\(^T\)) (Straub & Buchholz-Clevén, 2001), *Geobacter chapellei* (DSM 13688\(^T\)), *Geobacter grbicai* (ATCC BAA-45\(^T\)), *Geobacter hydrogenophilus* (ATCC 51590\(^T\) = DSM 13691\(^T\)) (Coates *et al.*, 2001), *Geobacter psychrophilus* (JCM 12644\(^T\)) and *Geobacter bemidjiensis* (ATCC BAA-1014\(^T\)) (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 β-subunit of RNA polymerase; *recA*, encoding the DNA repair protein, RecA; *gyrB*, the structural gene for the DNA gyrase β-subunit; *fusA*, encoding the protein synthesis elongation factor, elongation factor-Γ; *nifD*, encoding the ε-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) nitroacacetate, 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, 30, 37 and 42 ºC. Growth was evaluated using OD₆₀₀ values and microscopic examination. Growth occurred at 10, 17, 22, 30 and 37 ºC, with optimum growth at 30 ºC, 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 OD₆₀₀ 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,

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**Table 1. Physiological characteristics of Geobacter thiogenes strain K1T⁻¹ and Geobacter chapellei strain 172⁻¹**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Geobacter thiogenes strain K1T⁻¹</th>
<th>Geobacter chapellei strain 172⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA G+C content (mol%)</td>
<td>55.1</td>
<td>50.2</td>
</tr>
<tr>
<td>Temperature optimum (ºC)</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td>Temperature range (ºC)</td>
<td>10–37</td>
<td>NT</td>
</tr>
<tr>
<td>Donors:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acetoin (10 mM)</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Ethanol (10 mM)</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Formate (10 mM)</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Hydrogen (130 kPa)⁺</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Lactate (10 mM)</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Acceptors:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elemental sulfur (20 g l⁻¹)</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Fe(III) oxide (100 mM)</td>
<td>NT</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate (5 mM)</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Malate (10 mM)</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Mn(IV) oxide (20 g l⁻¹)</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Trichloroacetic acid (5 mM)</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

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

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Fig. 1. Growth of cells of G. thiogenes strain K1T⁻¹ in basal medium with Fe(III) nitroacacetate as electron acceptor and acetate or acetate plus hydrogen as electron donor.

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Reclassification of \textit{Trichlorobacter thiogenes}

Members of the family \textit{Geobacteraceae} have been detected or isolated from a wide variety of aquatic sediments and subsurface sediments. The ability of \textit{T. thiogenes} to grow via reductive dehalogenation was a novel characteristic for members of the \textit{Geobacter} cluster of the \textit{Geobacteraceae} for the capacity for dehalogenation has been observed previously in members of the \textit{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 \textit{Geobacter}, 'Geobacter lovleyi' strain SZ, is also capable of dechlorination (Sung et al., 2006). The finding that, like all \textit{Geobacter} species, \textit{T. thiogenes} is capable of Fe(III) reduction and contains abundant c-type cytochromes and that, using six separate genes, its phylogenetic placement within the \textit{Geobacter} clade of the \textit{Geobacteraceae} is maintained, indicates that \textit{T. thiogenes} should be reclassified as a species of \textit{Geobacter}.

\textbf{Description of \textit{Geobacter thiogenes} comb. nov.}

\textit{Geobacter thiogenes} (thi.o’ge.nes. Gr. n. thion sulfur; Gr. v. gennao produce; N.L. part. adj. thiogenes producing sulfur).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{phylogenetic_tree}
\caption{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 \textit{G. thiogenes} and other species within the family \textit{Geobacteraceae}. \textit{Desulfuromusa baki} was used as an outgroup. Bootstrap values were determined from 100 replicates. Bar, 0.05 nucleotide substitutions per site.}
\end{figure}

\textbf{Basonym: \textit{Trichlorobacter thiogenes}} De Wever et al. 2001b.

Physiological data and phylogenetic analysis based on nifD, recA, gyrB, rpoB, fusA and 16S rRNA genes indicate that \textit{Trichlorobacter thiogenes} is a member of the \textit{Geobacter} clade of the family \textit{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\textsuperscript{T} (= ATCC BAA-34\textsuperscript{T} = JCM 14045\textsuperscript{T}), which was enriched from subsoil from western Michigan with acetate as an electron donor and trichloroacetate acid as an electron acceptor.

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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.

\textbf{References}


