Reclassification of *Thermoterrabacterium ferrireducens* as *Carboxydotherrmus ferrireducens* comb. nov., and emended description of the genus *Carboxydotherrmus*

A. I. Slobodkin,¹ T. G. Sokolova,¹ A. M. Lysenko¹ and J. Wiegel²

Correspondence

A. I. Slobodkin

aslobodkin@hotmail.com

¹Winogradsky Institute of Microbiology, Russian Academy of Sciences, Prospect 60-letiya Oktyabrya 7/2, 117 312 Moscow, Russia

²Department of Microbiology, University of Georgia, Athens, GA 30602, USA

Similarities in phylogeny and metabolic properties between the type species of two monospecific genera of thermophilic anaerobic bacteria, *Carboxydotherrmus hydrogenoformans* and *Thermoterrabacterium ferrireducens*, and analysis of their recently available 16S rRNA gene sequences warranted clarification of their taxonomic positions. We have determined that the value of DNA–DNA hybridization between the type strains is 53 %. Additional physiological studies revealed that *C. hydrogenoformans* Z-2901^T is capable of Fe(III) reduction with H₂ as an electron donor and ferrihydrite as an electron acceptor. *T. ferrireducens* JW/AS-Y7^T is able to grow and utilize CO with ferrihydrite as an electron acceptor without hydrogen or acetate production. We therefore reclassify *Thermoterrabacterium ferrireducens* as *Carboxydotherrmus ferrireducens* comb. nov. (type strain JW/AS-Y7^T = DSM 11255^T = VKM B-2392^T). The description of the genus *Carboxydotherrmus* is emended to include such important physiological properties as growth on organic compounds and capacity for Fe(III) reduction.

Thermoterrabacterium ferrireducens JW/AS-Y7^T, a thermophilic, anaerobic, dissimilatory Fe(III)-reducing bacterium, was isolated from a hot spring in Yellowstone National Park (Slobodkin *et al.*, 1997). This micro-organism grew via utilization of organic substrates or molecular hydrogen and, besides ferric iron, reduced several other electron acceptors. 16S rRNA gene sequence analysis placed *T. ferrireducens* within the *Bacillus*–*Clostridium* subphylum, with the most closely related micro-organism at the time of description being *Ammonifex degensii* (83 % similarity). The combination of physiological properties and phylogenetic position allowed the organism to be assigned to a new genus.

The genus *Carboxydotherrmus* contains a single species, *Carboxydotherrmus hydrogenoformans*, an anaerobic carbon monoxide-utilizing thermophilic bacterium (Svetlichny *et al.*, 1991a). The name of this species was subsequently validly published (Svetlichny *et al.*, 1991b). A partial 16S rRNA gene sequence of *C. hydrogenoformans* Z-2901^T became available in 2000 (GenBank accession no. AF244579), and analysis revealed 98.4 % similarity between this sequence and that of *T. ferrireducens* JW/AS-Y7^T (GenBank accession no. U76363) (Sokolova *et al.*, 2001). The phylogenetic position of *C. hydrogenoformans* is confirmed by recently published whole-genome sequence data (Wu *et al.*, 2005; GenBank accession no. NC_007503).

This phylogenetic resemblance motivated Henstra & Stams (2004) to investigate the physiological properties of both micro-organisms in more detail. They found that the growth substrates for *C. hydrogenoformans* are not restricted to CO and pyruvate, as was previously reported (Pusheva & Sokolova, 1995), but that this bacterium is able to utilize various electron donors and acceptors for growth. The range of compounds utilized was identical to those used by *T. ferrireducens*. On the other hand, *T. ferrireducens* was capable of growth on CO with 9,10-anthraquinone 2,6-disulfonate or fumarate as electron acceptors (Henstra & Stams, 2004). Thus, the similarities in phylogeny and metabolic properties between *C. hydrogenoformans* and *T. ferrireducens* warranted a clarification of their taxonomic positions, as given below.

C. hydrogenoformans DSM 6008^T (=Z-2901^T) and *T. ferrireducens* DSM 11255^T (=JW/AS-Y7^T) were obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany) and routinely cultivated at 65 °C in mineral media as described previously (Svetlichny *et al.*, 1991a; Slobodkin *et al.*, 1997) with 100 % CO as the headspace gas (*C. hydrogenoformans*) and with the addition of glycerol (30 mM) and fumarate (20 mM) (*T. ferrireducens*). Growth of both micro-organisms in the presence of Fe(III) was investigated in anaerobic

bicarbonate-buffered medium (Slobodkin *et al.*, 1997) supplied with 0.2 g yeast extract l⁻¹ (Sigma). Ferrihydrite [poorly crystalline Fe(III) oxide] (90 mM), Fe(III) citrate (20 mM) or Fe(III) EDTA (15 mM) was provided as an electron acceptor and CO (100 % as headspace gas), H₂ (in the presence of CO₂ at a ratio of 80 : 20), formate (20 mM), lactate (20 mM) or glycerol (30 mM) was provided as an electron donor. In all experiments with different forms of Fe(III), the medium did not contain any reducing agents. Microscopy, cell counts and analysis of CO, acetate and Fe(II) were carried out as described elsewhere (Slobodkin *et al.*, 1997; Sokolova *et al.*, 2001). The DNA was extracted and purified by the method of Marmur (1961). DNA–DNA hybridization studies were performed by the optical reassociation method as described previously (Krivenko *et al.*, 1990).

The important physiological difference between *C. hydrogenoformans* and *T. ferrireducens* in CO metabolism is H₂ production by *C. hydrogenoformans* irrespective of the cultivation conditions. *T. ferrireducens* does not produce molecular hydrogen during CO utilization and is unable to grow on CO without an external electron acceptor. Growth of *C. hydrogenoformans* with Fe(III) and the ability of *T. ferrireducens* to utilize CO with Fe(III) as an electron acceptor have not been investigated previously. Our attempts to obtain sustainable growth of *C. hydrogenoformans* with either CO, glycerol or lactate as an electron donor and either ferrihydrite, Fe(III) citrate or Fe(III) EDTA as an electron acceptor did not yield positive results. The only electron donor/acceptor couple that supported growth and Fe(III) reduction by this organism was H₂/ferrihydrite. Under these conditions, within 7 days of cultivation, the maximal cell yield was $3.5\text{--}4.0 \times 10^7$ cells ml⁻¹, ferrihydrite was converted to a black magnetic precipitate with a Fe(II) content of 20–22 mM and no accumulation of acetate was found in the cultivation medium. Thus, the type species of the genus *Carboxydotherrmus* is capable of Fe(III) reduction. The inability of *C. hydrogenoformans* to grow on CO with Fe(III) is probably caused by the higher redox potential of the medium in comparison with the medium pre-reduced with Na₂S.9H₂O used in previous studies (Svetlichny *et al.*, 1991a; Henstra & Stams, 2004). As evident from growth on H₂/ferrihydrite, the hydrogenase of *C. hydrogenoformans* can work at higher redox potential than CO dehydrogenase. In contrast to *C. hydrogenoformans*, *T. ferrireducens* grew and utilized CO with ferrihydrite as an electron acceptor. After 7 days of cultivation, the maximal cell yield was $2.5\text{--}3.0 \times 10^7$ cells ml⁻¹, ferrihydrite was converted to a black magnetic precipitate with a Fe(II) content of 26–28 mM and 14 mM CO was consumed from the gas phase (45 % of the initial concentration) without producing acetate.

Subsequently, DNA–DNA hybridization of *T. ferrireducens* DSM 11255^T with *C. hydrogenoformans* DSM 6008^T revealed a reassociation value of 53 %. This level of relatedness is characteristic of different species belonging to the same genus (Johnson, 1984).

Considering the physiological similarities, phylogenetic position and level of DNA–DNA relatedness, *T. ferrireducens* and *C. hydrogenoformans* should be members of the same genus. According to the *Bacteriological Code* (Lapage *et al.*, 1992), the name *Carboxydotherrmus* has priority, thus *T. ferrireducens* has to be reclassified as a member of the genus *Carboxydotherrmus*. However, according to the original description (Svetlichny *et al.*, 1991a), representatives of the genus *Carboxydotherrmus* utilize CO with equimolar formation of H₂ and CO₂, do not utilize other organic and inorganic substrates and do not reduce sulfur. Therefore, taking into account the data obtained by Henstra & Stams (2004) and those generated in this study, the description of the genus *Carboxydotherrmus* needs to be emended.

Emended description of the genus *Carboxydotherrmus* Svetlichny *et al.* 1991

Rod-shaped, Gram-type positive (eu)bacteria. Anaerobic and thermophilic. Neutrophilic. Utilize CO either with or without production of molecular hydrogen. Able to couple the reduction of external electron acceptors such as Fe(III), sulfite, thiosulfate, elemental sulfur, nitrate, fumarate or 9,10-anthraquinone 2,6-disulfonate with oxidation of organic acids, polyols and H₂. In the presence as well as in the absence of an electron acceptor, organic substrates are oxidized incompletely to acetate as the main metabolic product. Do not reduce sulfate.

Description of *Carboxydotherrmus ferrireducens* comb. nov.

Carboxydotherrmus ferrireducens [fer.ri.re.du'cens. L. n. ferrum iron; L. part. adj. *reducens* converting to a different state; N.L. part. adj. *ferrireducens* reducing (ferric) iron].

Basonym: *Thermoterrabacterium ferrireducens* Slobodkin *et al.* 1997.

The description is based on that published previously for *Thermoterrabacterium ferrireducens* (Slobodkin *et al.*, 1997) with the additional data obtained by Gavrilov *et al.* (2003), Henstra & Stams (2004) and during this study. Cells are straight to slightly curved rods, 0.3–0.4 µm in diameter and 1.6–2.7 µm in length with rounded ends. Cells stain Gram-positive. Cells occur singly or in pairs, with retarded peritrichous flagellation, and exhibit tumbling motility. Spores are not observed. Anaerobic. Grows in mineral medium without supplementation with complex media components. The temperature range for growth is 50–74 °C with an optimum at 65 °C. The pH^{65 °C} range for growth is 5.5–7.6, with an optimum at 6.0–6.2. No growth was observed at or below 48 °C, at or above 76 °C, at or below pH^{65 °C} 5.3 and at or above pH^{65 °C} 7.8. Growth occurs at NaCl concentrations in the range of 0–1.0 % (w/v). In the presence of CO₂, the type strain is able to grow with glycerol as the only organic carbon source. Couples the oxidation of glycerol to reduction of ferrihydrite [poorly crystalline Fe(III) oxide], Fe(III) citrate or Fe(III) EDTA as electron

acceptors. In the presence as well as in the absence of Fe(III) and in the presence of CO₂, glycerol is oxidized incompletely to acetate as the only organic metabolic product, leading to a ratio of more than one acetate molecule produced per glycerol molecule consumed. Does not produce H₂. Utilizes glycerol, lactate, 1,2-propanediol, glycerate, pyruvate, yeast extract and peptone. Grows weakly on glucose, fructose and mannose, producing acetate as the only organic product. In the presence of ferrihydrite, grows lithoautotrophically with molecular hydrogen as an electron donor and CO₂ as the only carbon source. Utilizes CO with ferrihydrite, fumarate or 9,10-anthraquinone 2,6-disulfonate as electron acceptors, without the production of molecular hydrogen or acetate. No growth occurs with acetate, methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, propionate, acetone, ethylene glycol, 1,3-propanediol, fumarate, succinate, phenol, benzoate, 9,10-anthraquinone 2,6-disulfonate, starch, olive oil, elemental sulfur, sucrose, galactose, xylose, cellobiose or arabinose with or without Fe(III). Reduces Fe(III) to Fe(II), 9,10-anthraquinone 2,6-disulfonate to 9,10-anthrahydroquinone 2,6-disulfonate, fumarate to succinate, thiosulfate to elemental sulfur or sulfide, sulfite to sulfide, elemental sulfur to sulfide and nitrate to nitrite and ammonium. Does not reduce MnO₂ or sulfate. Growth is inhibited by chloramphenicol, erythromycin and rifampicin at 100 µg ml⁻¹ but not by 100 µg ampicillin, streptomycin or tetracycline ml⁻¹. The DNA base composition is 41 mol% G + C (HPLC).

The type strain is JW/AS-Y7^T (=DSM 11255^T = VKM B-2392^T), isolated from a freshwater hot spring in the Calcite Springs area of Yellowstone National Park, WY, USA.

Acknowledgements

This work was supported by the programs 'Molecular and Cell Biology' and 'Origin and Evolution of Life' of the Russian Academy of Sciences and by grant 06-04-48684-a from the Russian Foundation for Basic Research.

References

Gavrilov, S. N., Bonch-Osmolovskaya, E. A. & Slobodkin, A. I. (2003). Physiology of organotrophic and lithotrophic growth of the thermophilic iron-reducing bacteria *Thermoterrabacterium*

ferrireducens and *Thermoanaerobacter siderophilus*. *Microbiology* (English translation of *Mikrobiologiya*) **72**, 132–137.

Henstra, A. M. & Stams, A. J. M. (2004). Novel physiological features of *Carboxydotherrnus hydrogenoformans* and *Thermoterrabacterium ferrireducens*. *Appl Environ Microbiol* **70**, 7236–7240.

Johnson, J. L. (1984). Nucleic acids in bacterial classification. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 8–11. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.

Krivenko, V. V., Vadachkoriya, R. M., Chernykh, N. A., Mityushina, L. L. & Krassilnikova, E. N. (1990). *Clostridium uzonii* sp. nov., an anaerobic thermophilic glycolytic bacterium from the hot springs of Kamchatka. *Microbiology* (English translation of *Mikrobiologiya*) **59**, 741–749.

Lapage, S. P., Sneath, P. H. A., Lessel, E. F., Skerman, V. B. D., Seeliger, H. P. R. & Clark, W. A. (editors) (1992). *International Code of Nomenclature of Bacteria* (1990 Revision). *Bacteriological Code*. Washington, DC: American Society for Microbiology.

Marmur, J. (1961). A procedure for the isolation of desoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

Pusheva, M. A. & Sokolova, T. G. (1995). Distribution of CO-dehydrogenase activity in anaerobic thermophilic carboxydophilic bacterium *Carboxydotherrnus hydrogenoformans* grown at the expense of CO or pyruvate. *Microbiology* (English translation of *Mikrobiologiya*) **64**, 491–495.

Slobodkin, A. I., Reysenbach, A.-L., Strutz, N., Dreier, M. & Wiegel, J. (1997). *Thermoterrabacterium ferrireducens* gen. nov., sp. nov. a thermophilic anaerobic, dissimilatory Fe(III)-reducing bacterium from a continental hot spring. *Int J Syst Bacteriol* **47**, 541–547.

Sokolova, T. G., Gonzalez, J. M., Kostrikina, N. A., Chernykh, N. A., Tourova, T. P., Kato, C., Bonch-Osmolovskaya, E. A. & Robb, F. T. (2001). *Carboxydotherrnus pacificum* gen. nov., sp. nov., a new anaerobic, thermophilic, CO-utilizing marine bacterium from Okinawa Trough. *Int J Syst Evol Microbiol* **51**, 141–149.

Svetlichny, V. A., Sokolova, T. G., Gerhardt, M., Ringpfeil, M., Kostrikina, N. A. & Zavarzin, G. A. (1991a). *Carboxydotherrnus hydrogenoformans* gen. nov., sp. nov., a CO-utilizing thermophilic anaerobic bacterium from hydrothermal environments of Kunashir Island. *Syst Appl Microbiol* **14**, 245–260.

Svetlichny, V. A., Sokolova, T. G., Gerhardt, M., Ringpfeil, M., Kostrikina, N. A. & Zavarzin, G. A. (1991b). *Carboxydotherrnus hydrogenoformans* gen. nov., sp. nov. In *Validation of the Publication of New Names and New Combinations Previously Effectively Published Outside the IJSB*, List no. 39. *Int J Syst Bacteriol* **41**, 580–581.

Wu, M., Ren, Q., Durkin, A. S. & 14 other authors (2005). Life in hot carbon monoxide: the complete genome sequence of *Carboxydotherrnus hydrogenoformans* Z-2901. *PLoS Genet* **1**, e65.