Desulfotomaculum ferrireducens sp. nov., a moderately thermophilic sulfate-reducing and dissimilatory Fe(III)-reducing bacterium isolated from compost

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A novel dissimilatory Fe(III)-reducing bacterium, designated strain GSS09^T, was isolated from a compost sample by using a solid medium containing acetate and ferrihydrite as electron donor and electron acceptor, respectively. Cells of strain GSS09^T were anaerobic, Gram-stain-positive, motile, endospore-forming and rod-shaped. Growth occurred at 30–55 °C (optimum 50 °C), at pH 6.5–9.0 (optimum pH 7.5) and in the presence of 0–3 % (w/v) NaCl (optimum 1 %). Both sulfur compounds such as sulfate, sulfite and thiosulfate and Fe(III) oxides such as ferrihydrite could be utilized as electron acceptors. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain GSS09^T was related closely to *Desulfotomaculum hydrothermale* Lam5^T (94.5 % sequence similarity). The major fatty acids were $C_{16:0}$ and $C_{16:1}\omega7c$/$C_{16:1}\omega6c$. The G+C content of the genomic DNA was 49.1 mol%. On the basis of phylogenetic analysis, phenotypic characterization and physiological tests, strain GSS09^T is considered to represent a novel species of the genus *Desulfotomaculum*, for which the name *Desulfotomaculum ferrireducens* sp. nov. is proposed. The type strain is GSS09^T (=KCTC 15523^T=MCCC 1K01254^T).

The genus *Desulfotomaculum* of the order *Clostridiales* in the phylum *Firmicutes* (Gibbons & Murray, 1978) was first described by Campbell & Postgate (1965), with *Desulfotomaculum nigrificans* as the type species (Werkman & Weaver, 1927; Campbell & Postgate, 1965). At the time of writing, 29 species with validly published names in the genus *Desulfotomaculum* have been isolated from various environments (http://www.bacterio.net/desulfotomaculum.html), after *Desulfotomaculum guttoideum* was moved to the genus *Clostridium* (Stackebrandt *et al.*, 1997), and *Desulfotomaculum orientis* and *Desulfotomaculum auripigmentum* were moved to the genus *Desulfosporosinus* (Stackebrandt *et al.*, 1997, 2003). According to phylogenetic data, species of the genus *Desulfotomaculum* exhibit unusual differences in their 16S rRNA gene sequences resulting in the formation of six well-separated subclusters, Ia–If (Stackebrandt *et al.*, 1997; Ogg & Patel, 2011). Phenotypically, members of the genus *Desulfosporosinus* are obligately anaerobic, endospore-forming, motile and rod-shaped (Campbell & Postgate, 1965). The genus *Desulfotomaculum* comprises thermophilic and mesophilic bacteria, and Gram-stain reactions for these bacteria are variable: Gram-staining for all members of the valid mesophilic group such as *Desulfotomaculum halophilum* and *Desulfotomaculum defluvii* is negative (Jacquenod *et al.*, 1998; Krishnamurthi *et al.*, 2013) while for most members of the thermophilic group such as *Desulfotomaculum thermocisternum* and *Desulfotomaculum peckii* it is positive (Nilsen *et al.*, 1996; Jabari *et al.*, 2013).

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Abbreviations: AQDS, anthraquinone-2, 6-disulphonate; IRB, iron-reducing bacteria; SRB, sulfate-reducing bacteria.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain GSS09^T is KU589293.

Three supplementary figures are available with the online Supplementary Material.

Sulfate-reducing bacteria (SRB) have a large role in the biogeochemical sulfur cycle, and most described thermophilic SRB belong to the genus *Desulfotomaculum* (Goorissen *et al.*, 2003; Kaksonen *et al.*, 2006). Dissimilatory microbial Fe(III) reduction is an important terminal electron accepting process coupled to the oxidation of organic matter in anaerobic environments (Lovley *et al.*, 2004), and iron-reducing bacteria (IRB) have been found to be involved in the bioremediation of organic pollutants and heavy metals (Wielinga *et al.*, 2001; Wilkins *et al.*, 2006). To date, strain MI-1 belonging to the invalidly named species '*Desulfotomaculum reducens*' is the only SRB of the genus *Desulfotomaculum* that is able to grow with Fe(III) as sole electron acceptor under moderate temperature (Tebo & Obraztsova, 1998).

In this study, a moderately thermophilic bacterium, designated strain GSS09^T, capable of growing by gaining energy from dissimilatory Fe(III) oxide reduction, was isolated and characterized. Based on its phylogenetic position and phenotypic, physiological and chemotaxonomic characteristics, strain GSS09^T is proposed to represent a novel species of the genus *Desulfotomaculum*. This organism is the first thermophilic bacterium in the genus *Desulfotomaculum* that shares properties with both sulfate-reducing and dissimilatory Fe(III)-reducing bacteria.

The sample for isolation of strain GSS09^T was obtained from a composting demonstration plant in Dongguan City, Guangdong Province, China (23.04° N 113.75° E), as described by Yang *et al.* (2015). For enrichment, 10 g of soil was added to a sterile bottle containing 50 ml sterilized mineral salts medium [MSM, containing (per litre) 0.6 g NaH_2PO_4, 0.25 g NH_4Cl, 0.1 g KCl, 2.5 g $NaHCO_3$, 10.0 ml vitamin stock solution and 10.0 ml mineral stock solution (Li *et al.*, 2009), pH 7.2] supplemented with 25 mM ferrihydrite and 10 mM acetate as electron acceptor and donor, respectively. The bottle was purged with N_2/CO_2 (80 : 20, v/v) for 30 min, sealed with a butyl-rubber stopper and an aluminium cap and incubated at 50 °C. After the colour of the soil suspension changed to dark grey, the enriched culture of 5 ml was transferred into 50 ml of fresh MSM supplemented with 25 mM ferrihydrite and 10 mM acetate and incubated at 50 °C as described above. This procedure was repeated three times. To obtain a pure culture, the enriched population was serially diluted and spread onto MSM agar containing 20 g agar l^{-1}, 25 mM ferrihydrite and 10 mM acetate at 50 °C in an anaerobic chamber (ShelLab; Sheldon Manufacturing). After 15 days of incubation on the agar plate, single colonies were picked and streaked on the same agar for repeated purification. One strain, designated GSS09^T, was finally selected and used for further studies.

In the present study, the characteristics of strain GSS09^T were investigated by using a detailed polyphasic taxonomic investigation. Based on phylogenetic analysis of the 16S rRNA gene sequence, *Desulfotomaculum hydrothermale* DSM 18033^T, *Desulfotomaculum aeronauticum* DSM 10349^T, *D. defluvii* DSM 23699^T and *Desulfotomaculum ruminis* DSM 2154^T were selected as reference strains. These reference strains were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany) and were cultured under the conditions recommended by the DSMZ.

To analyse the phylogenetic position of strain GSS09^T, genomic DNA was extracted using a DNA extraction kit (Aidlab). The 16S rRNA gene was amplified by PCR using the universal primers 27F and 1492R (Lane *et al.*, 1985). The PCR product was gel-purified using a D2500-01 Gel Extraction Kit (Omega Bio-tek), cloned into plasmid vector using a TA cloning kit (TaKaRa) and then checked by sequencing both strands. Pairwise sequence similarities were calculated using the EzTaxon-e server (http://eztaxon-e.ezbiocloud.net/; Kim *et al.*, 2012). Phylogenetic analyses were carried out using MEGA version 6.0 (Tamura *et al.*, 2013). Distances were calculated using distance options according to the maximum composite likelihood model and clustering was performed with the neighbour-joining and maximum-likelihood methods. Statistical support for the branches of the phylogenetic trees was determined using bootstrap analysis (based on 1000 re-samplings) (Felsenstein, 1985).

The 16S rRNA gene sequence of strain GSS09^T comprised 1537 nt and was most closely related to *D. hydrothermale* Lam5^T (94.5 % similarity) and '*D. reducens*' MI-1 (93.9 %). The phylogenetic trees reconstructed using the neighbour-joining and maximum-likelihood methods grouped strain GSS09^T as a member of the genus *Desulfotomaculum* and clearly placed the new isolate in a distinct phylogenetic lineage with '*D. reducens*', *D. aeronauticum*, *D. defluvii* and *D. ruminis* of *Desulfotomaculum* subcluster Ia defined previously (Stackebrandt *et al.*, 1997) (Figs 1 and S1, available in the online Supplementary Material).

For cell morphology observation with a transmission electron microscope (JEM 1400; JEOL), cells were grown in MSM supplemented with 10 mM sulfite as electron acceptor and 20 mM pyruvate as electron donor for 2 days. In preparation for electron microscopy, cells were suspended in 0.85 % (w/v) NaCl, dried on a nickel-coated mesh and negatively stained with phosphotungstic acid. Endospores were observed by transmission electron microscopy after cells were grown for 5 days according to Vaz-Moreira *et al.* (2012). Colonies were observed on MSM agar with sulfite as electron acceptor and pyruvate as electron donor after incubation at 50 °C for 7 days. The Gram reaction was performed using a Gram staining kit (HB8278; Qingdao Hope Bio-Technology), and the cell-wall structure was confirmed by the KOH lysis test using 3 % (w/v) KOH. To investigate the temperature range for growth, the isolate was grown at 20, 25, 30, 37, 40, 42, 45, 50, 55 and 60 °C. Growth was tested at pH 5.0–10.0 at intervals of 0.5 pH units, and NaCl tolerance was examined at a range of 0–6 % (w/v) NaCl concentrations with increments of 0.5 %. Utilization of electron donors (20 mM) including

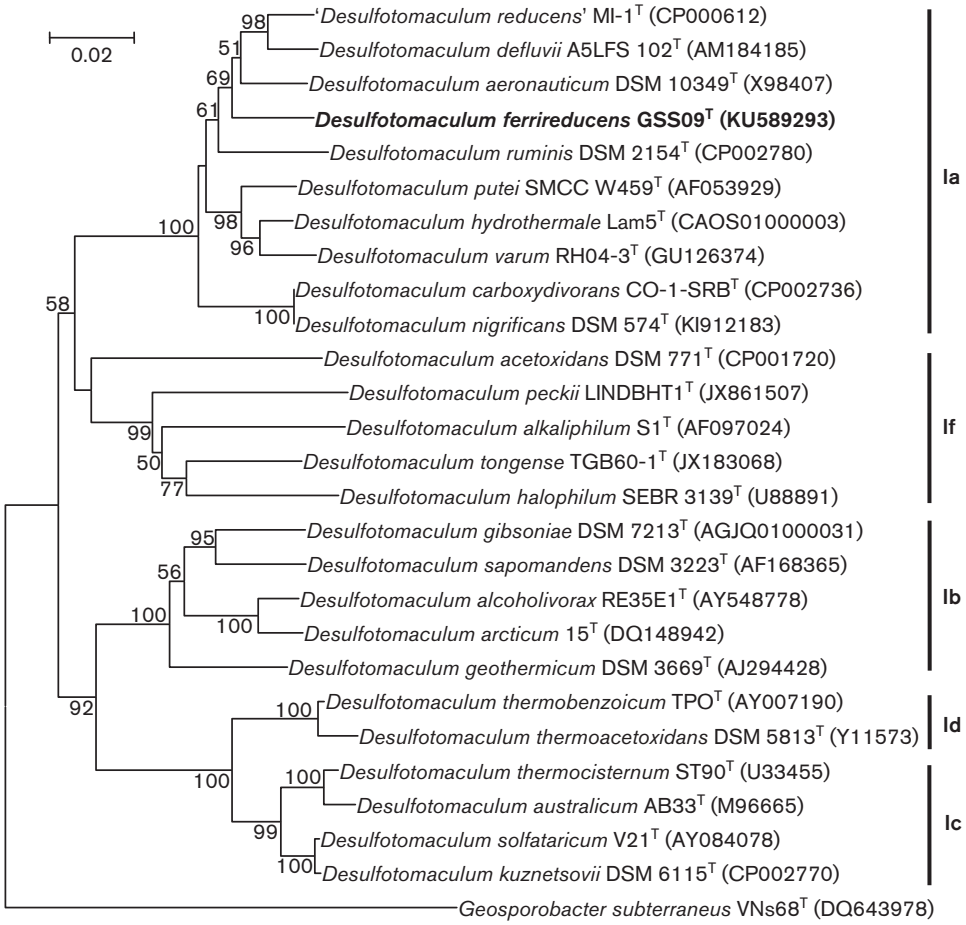

Fig. 1. Neighbour-joining tree based on 16S rRNA gene sequences showing the phylogenetic position of strain GSS09^T and representatives of some other related taxa. *Geosporobacter subterraneus* VNs68^T was selected as the outgroup. Bootstrap values (expressed as percentages of 1000 replications) of ≥50 % are shown at branch points. Bar, 0.02 substitutions per nucleotide position.

xylose, glucose, malate, formate, acetate, lactate, ethanol, sucrose, pyruvate, fructose, glycerol, lactose, succinate and fumarate was examined at 50 °C in the presence of 10 mM sulfite. Fermentative growth using the above substrates was also investigated in the absence of an electron acceptor. Utilization of electron acceptors (10 mM) including sulfite, ferrihydrite, sulfate, nitrate, nitrite, thiosulfate and elemental sulfur was determined in the presence of 20 mM pyruvate. Each substrate was studied in three parallel replicates.

Strain GSS09^T was anaerobic. Cells were rod-shaped, 3.5–4.6 μm in length and 0.8–1.1 μm in diameter, and endospore-forming (Fig. S2). Small and grey-pigmented colonies were observed. The cells stained Gram-positive, and the KOH lysis test suggested that the cell wall of the isolate has a Gram-positive structure. Growth occurred at 30–55 °C and pH 6.5–9.0, and the optimum temperature and pH for growth were 50 °C and pH 7.5. The novel strain utilized xylose, glucose, formate, acetate, lactate, ethanol, sucrose, pyruvate, fructose, glycerol and lactose as electron donors with sulfite as electron acceptor. Fermentative growth was observed using fructose or pyruvate. The main product resulting from fructose and pyruvate fermentation was acetate. With pyruvate as electron donor, strain GSS09^T was able to reduce sulfite, sulfate and thiosulfate to hydrogen sulfide, reduce nitrate and nitrite to ammonium, and reduce ferrihydrite to magnetite; elemental sulfur could not be reduced. The detailed phenotypic features of strain GSS09^T and differences in several phenotypic properties with respect to its closest phylogenetic relatives are included in the species description and Table 1.

The reduction of 50 mM ferrihydrite by strain GSS09^T was monitored with 20 mM pyruvate as electron donor in the presence and absence of the soluble electron carrier anthraquinone-2, 6-disulphonate (AQDS; 0.2 mM). Total Fe(II) was extracted with 0.5 mM HCl for 1.5 h and colorimetrically quantified using 1,10-phenanthroline as described by Wu *et al.* (2010). As shown in Fig. S3, strain GSS09^T reduced about 35 % of the Fe(III) after incubation

Table 1. Characteristics of strain GSS09^T and the type strains of closely related *Desulfotomaculum* species

Strains: 1, GSS09^T; 2, *D. hydrothermale* DSM 18033^T; 3, *D. aeronauticum* DSM 10349^T; 4, *D. defluvii* DSM 23699^T; 5, *D. ruminis* DSM 2154^T; 6, '*D. reducens*' MI-1 (Tebo & Obraztsova, 1998; Junier *et al.*, 2010a,b). Data were from this study unless indicated otherwise. Electron acceptors were determined using pyruvate as electron donor for strains GSS09^T, *D. hydrothermale* DSM 18033^T and *D. aeronauticum* DSM 10349^T, and using lactate as electron donor for *D. defluvii* DSM 23699^T and *D. ruminis* DSM 2154^T; electron donors were determined using sulfite as electron acceptor for all strains. All strains were endospore-forming, and were able to utilize sulfite and thiosulfate as electron acceptors. In the case of electron donors, all strains utilized ethanol. +, Positive; −, negative; ND, not determined.

Characteristic	1	2	3	4	5	6
Cell shape	Rod	Curved rod	Curved rod	Rod	Rod	Curved rod
Cell size (μm)	0.8–1.1× 3.5–4.6	0.5× 2.0–5.0	0.5–0.8× 2.0–5.5	0.5–0.7× 2.0–4.0	0.5–0.7× 2.2–4.0	0.8–1.0× 5–10
Motility	+	+	+	−	+	+
Gram staining reaction	+	−	−	−	−	+
Temperature range (°C)	30–55	37–60	20–45	25–45	25–45	ND
Optimum temperature (°C)	50	55	37	37	37	37
pH range	6.5–9.0	5.8–8.2	6.0–9.0	6.5–8.5	6.0–8.0	ND
Optimum pH	7.5	7.1	7.0	7.5	7.5	7.0–7.2
NaCl tolerance (%, w/v)	0–3.0	0–1.5	0–2.5	0–2.0	0–2.0	ND
Electron donors:						
Formate	+	+	+	−	+	+
Lactate	+	+	−	+	+	+
Acetate	+	−	−	+	−	−
Fumarate	−	−	−	+	−	−
Pyruvate	+	+	+	−	+	+
Glucose	+	−	−	+	−	+
Sucrose	+	−	−	−	−	ND
Fructose	+	−	−	+	−	ND
Glycerol	+	+	+	−	+	ND
Xylose	+	−	−	+	−	ND
Lactose	+	−	−	−	−	ND
Electron acceptors:						
Sulfate	+	+	−	+	+	+
Sulfur	−	−	−	−	−	+
Nitrate	+	−	−	−	−	+
DNA G+C content (mol%)*	49.1	46.8[a]	43.8[b]	45.4[c]	45.5[d]	43.28

*Data were taken from: *a*, Haouari *et al.* (2008); *b*, Hagenauer *et al.* (1997); *c*, Krishnamurthi *et al.* (2013); *d*, Campbell *et al.* (1965).

for 10 days, and slightly more Fe(II) during incubation from 10 to 20 days. The addition of AQDS significantly stimulated the reduction of ferrihydrite, permiting the greater amounts of total Fe(II) in the culture. This result was similar to the report for another Gram-positive thermophilic IRB, *Carboxydothermus ferrireducens* DSM 11255 (Gavrilov *et al.*, 2012).

For cellular fatty acid analysis, cells of strain GSS09^T and the reference strains were grown in MSM containing 10 mM sulfite and 20 mM pyruvate (pyruvate was replaced by lactate for *D. defluvii* DSM 23699^T and *D. ruminis* DSM 2154^T) at 40 °C. Cells were collected and fatty acids in whole cells were saponified, methylated and extracted according to the standard protocol of MIDI (Sherlock Microbial Identification System, version 6.0B). The fatty acids were analysed by GC (Agilent Technologies 6850) and identified using the TSBA6.0 database of the Microbial Identification System (Sasser, 1990). The G+C content of the genomic DNA was determined by HPLC according to the method described by Mesbah *et al.* (1989).

As shown in Table 2, strain GSS09^T contained $C_{16:0}$ (32.9 %) as the predominant fatty acid; this fatty acid was present at much lower amounts in *D. hydrothermale* DSM 18033^T (19.5 %) and *D. ruminis* DSM 2154^T (14.5 %). The fatty acid profile of strain GSS09^T also displayed other significant differences from its phylogenetic relatives, comprising much less iso-$C_{15:0}$ (6.8 %) than that in *D. hydrothermale* DSM 18033^T (18.7 %), *D. aeronauticum* DSM 10349^T (13.4 %) and, in particular, *D. ruminis* DSM 2154^T (40.8 %), and less $C_{16:1}\omega7c/C_{16:1}\omega6c$ (13.8 %) than that in *D. defluvii* DSM

Table 2. Cellular fatty acid profiles of strain GSS09^T and related species of the genus *Desulfotomaculum*

Strains: 1, GSS09^T; 2, *D. hydrothermale* DSM 18033^T; 3, *D. aeronauticum* DSM 10349^T; 4, *D. defluvii* DSM 23699^T; 5, *D. ruminis* DSM 2154^T. Data were taken from this study. Values are percentages of the total fatty acids. –, Not detected or <1 %.

Fatty acid	1	2	3	4	5
Saturated straight-chain					
$C_{12:0}$	–	–	–	2.7	–
$C_{14:0}$	3.7	2.7	5.3	9.2	4.1
$C_{14:0}$ 2-OH	–	–	–	–	1.3
$C_{16:0}$	32.9	19.5	29.7	26.8	14.5
$C_{17:0}$	2.4	1.1	–	–	–
$C_{18:0}$	3.7	–	6.9	1.2	1.2
Unsaturated straight-chain					
$C_{15:1}\omega 8c$	–	–	–	1.3	–
$C_{16:1}\omega 9c$	4.6	2.4	4.1	10.7	1.3
$C_{16:1}\omega 5c$	–	–	–	1.7	–
$C_{17:0}$ cyclo	–	4.7	–	–	1.3
$C_{17:1}\omega 6c$	1.2	–	–	–	–
$C_{17:1}\omega 8c$	1.2	–	–	–	–
$C_{18:1}\omega 5c$	–	–	–	1.2	–
$C_{18:1}\omega 9c$	–	–	2.8	3.0	–
Saturated branched-chain					
iso-$C_{14:0}$ 3-OH	–	–	–	–	3.8
iso-$C_{15:0}$	6.8	18.7	13.4	–	40.8
anteiso-$C_{15:0}$	2.0	3.9	4.0	–	11.9
iso-$C_{16:0}$	1.1	1.2	–	–	–
iso-$C_{17:0}$	4.4	11.7	9.2	–	6.1
anteiso-$C_{17:0}$	1.3	2.7	2.6	–	1.4
iso-$C_{15:1}$ F	–	1.07	–	–	–
iso-$C_{19:1}$ I	1.1	–	–	–	–
Unsaturated branched-chain					
Summed feature 3*	13.8	4.7	7.3	26.7	1.3
Summed feature 4*	6.2	5.5	9.0	2.8	3.6
Summed feature 8*	2.7	2.0	–	7.9	2.7
Summed feature 9*	3.6	8.6	–	–	–

*Summed features are groups of two or three fatty acids that cannot be separated by GLC using the MIDI system. Summed feature 3 comprises $C_{16:1}\omega 7c$ and/or $C_{16:1}\omega 6c$; summed feature 4 comprises iso-$C_{17:1}$ I and/or anteiso-$C_{17:1}$ B; summed feature 8 comprises $C_{18:1}\omega 7c$ and/or $C_{18:1}\omega 6c$; summed feature 9 comprises iso-$C_{17:1}\omega 9c$ and/or $C_{16:0}$ 10-methyl.

23699^T (26.7 %). The genomic DNA G+C content of the new isolate was 49.1 mol%.

In summary, the phenotypic and phylogenetic analyses suggested that strain GSS09^T was a member of the genus *Desulfotomaculum*. However, the new isolate showed low 16S rRNA gene sequence similarities with the type strains of other known species of this genus. In addition, the new isolate can be distinguished from its closest relatives based on several characteristics such as Gram-staining reaction, temperature range and optimum temperature for growth, electron donors/acceptors and fatty acid profiles. Based on these differences, strain GSS09^T is considered to represent a novel species of the genus *Desulfotomaculum*, for which the name *Desulfotomaculum ferrireducens* sp. nov. is proposed.

Description of *Desulfotomaculum ferrireducens* sp. nov.

Desulfotomaculum ferrireducens [fer.ri.re.du′cens. L. n. *ferrum* iron; L. part. adj. *reducens* leading back, bringing back and in chemistry converting to a different oxidation state; N.L. part. adj. *ferrireducens* reducing Fe(III) to Fe(II)].

Cells are Gram-stain-positive, strictly anaerobic, flagellum-forming and rod-shaped, 0.8–1.1×3.5–4.6 μm in size. Cylindrical or ellipsoidal endospores are formed at subterminal or terminal position, in a non-swollen sporangium. Growth occurs at 30–55 °C (optimum 50 °C), at pH 6.5–9.0 (optimum pH 7.5) and in the presence of 0–3 % (w/v) NaCl (optimum 1 %). With sulfite as an electron acceptor, xylose, glucose, formate, acetate, lactate, ethanol, sucrose,

pyruvate, fructose, glycerol and lactose can be utilized as electron donors, but malate, succinate and fumarate cannot be utilized. With pyruvate as an electron donor, ferrihydrite, sulfite, sulfate, nitrate, nitrite and thiosulfate (but not elemental sulfur) can be reduced. Fructose and pyruvate can be fermented for cell growth. The major fatty acids are $C_{16:0}$ and $C_{16:1}\omega7c$/$C_{16:1}\omega6c$.

The type strain, GSS09^T (=KCTC 15523^T=MCCC 1K01254^T), was isolated from a compost sample in Dongguan city, Guangdong Province, China. The G+C content of the genomic DNA of the type strain is 49.1 mol%.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (41301257), the Science and Technology Planning Project of Guangdong Province (2013B060400042 and 2014A030304052), and the Science and Technology Planning Project of Guangzhou City (2014Y2-00194).

References

Campbell, L. L. & Postgate, J. R. (1965). Classification of the spore-forming sulfate-reducing bacteria. *Bacterio Rev* **29**, 359–363.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Gavrilov, S. N., Lloyd, J. R., Kostrikina, N. A. & Slobodkin, A. I. (2012). Fe(III) oxide reduction by a gram-positive thermophile: physiological mechanisms for dissimilatory reduction of poorly crystalline fe(III) oxide by a thermophilic gram-positive bacterium *Carboxydothermus ferrireducens*. *Geomicrobiol J* **29**, 804–819.

Gibbons, N. E. & Murray, R. G. E. (1978). Proposals concerning the higher taxa of bacteria. *Int J Syst Bacteriol* **28**, 1–6.

Goorissen, H. P., Boschker, H. T., Stams, A. J. & Hansen, T. A. (2003). Isolation of thermophilic *Desulfotomaculum* strains with methanol and sulfite from solfataric mud pools, and characterization of *Desulfotomaculum solfataricum* sp. nov. *Int J Syst Evol Microbiol* **53**, 1223–1229.

Hagenauer, A., Hippe, H. & Rainey, F. A. (1997). *Desulfotomaculum aeronauticum* sp. nov., a sporeforming, thiosulfate-reducing bacterium from corroded Aluminium alloy in an aircraft. *Syst Appl Microbiol* **20**, 65–71.

Haouari, O., Fardeau, M. L., Cayol, J. L., Casiot, C., Elbaz-Poulichet, F., Hamdi, M., Joseph, M. & Ollivier, B. (2008). *Desulfotomaculum hydrothermale* sp. nov., a thermophilic sulfate-reducing bacterium isolated from a terrestrial Tunisian hot spring. *Int J Syst Evol Microbiol* **58**, 2529–2535.

Jabari, L., Gannoun, H., Cayol, J., Hamdi, M., Ollivier, B., Fauque, G. & Fardeau, M. (2013). *Desulfotomaculum peckii* sp. nov., a moderately thermophilic member of the genus *Desulfotomaculum*, isolated from an upflow anaerobic filter treating abattoir wastewaters. *Int J Syst Evol Microbiol* **63**, 2082–2087.

Jacquenod, C., Magot, M., Patel, B. K., Matheron, R. & Caumette, P. (1998). *Desulfotomaculum halophilum* sp. nov., a halophilic sulfate-reducing bacterium isolated from oil production facilities. *Int J Syst Bacteriol* **48**, 333–338.

Junier, P., Junier, T., Podell, S., Sims, D. R., Detter, J. C., Lykidis, A., Han, C. S., Wigginton, N. S., Gaasterland, T. & Bernier-Latmani, R. (2010a). The genome of the Gram-positive metal- and sulfate-reducing bacterium *Desulfotomaculum reducens* strain MI-1. *Environ Microbiol* **12**, 2738–2754.

Junier, P., Suvorova, E. I. & Bernier-Latmani, R. (2010b). Effect of competing electron acceptors on the reduction of U(VI) by *Desulfotomacilum reducens*. *Geomicroiol J* **27**, 435–443.

Kaksonen, A. H., Spring, S., Schumann, P., Kroppenstedt, R. M. & Puhakka, J. A. (2006). *Desulfotomaculum thermosubterraneum* sp. nov., a thermophilic sulfate-reducer isolated from an underground mine located in a geothermally active area. *Int J Syst Evol Microbiol* **56**, 2603–2608.

Kim, O. S., Cho, Y. J., Lee, K., Yoon, S. H., Kim, M., Na, H., Park, S. C., Jeon, Y. S., Lee, J. H. & other authors (2012). Introducing EzTaxon-e: a prokaryotic 16S rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716–721.

Krishnamurthi, S., Spring, S., Kumar, P. A., Mayilraj, S., Klenk, H. P. & Suresh, K. (2013). *Desulfotomaculum defluvii* sp. nov., a sulfate-reducing bacterium isolated from the subsurface environment of a landfill. *Int J Syst Evol Microbiol* **63**, 2290–2295.

Lane, D. J., Pace, B., Olsen, G. J., Stahl, D. A., Sogin, M. L. & Pace, N. R. (1985). Rapid determination of 16S ribosomal RNA sequences for phylogenetic analyses. *Proc Natl Acad Sci U S A* **82**, 6955–6959.

Li, X. M., Zhou, S. G., Li, F. B., Wu, C. Y., Zhuang, L., Xu, W. & Liu, L. (2009). Fe(III) oxide reduction and carbon tetrachloride dechlorination by a newly isolated *Klebsiella pneumoniae* strain L17. *J Appl Microbiol* **106**, 130–139.

Lovley, D. R., Holmes, D. E. & Nevin, K. P. (2004). Dissimilatory Fe(III) and Mn(IV) reduction. *Adv Microbial Physiol* **49**, 219–286.

Mesbah, M., Premachandran, U. & Whitman, W. B. (1989). Precise measurement of the G+C content of deoxyribonucleic acid by high-performance liquid chromatography. *Int J Syst Bacteriol* **39**, 159–167.

Nilsen, R. K., Torsvik, T. & Lien, T. (1996). *Desulfotomaculum thermocisternum* sp. nov., a sulfate reducer isolated from a hot North Sea oil reservoir. *Int J Syst Bacteriol* **46**, 397–402.

Ogg, C. D. & Patel, B. K. (2011). *Desulfotomaculum varum* sp. nov., a moderately thermophilic sulfate-reducing bacterium isolated from a microbial mat colonizing a great artesian basin bore well runoff channel. *3 Biotech* **1**, 139–149.

Sasser, M. (1990). Identification of bacteria by gas chromatography of cellular fatty acids. *MIDI Technical Note 101*. Newark, DE: MIDI.

Stackebrandt, E., Sproer, C., Rainey, F. A., Burghardt, J., Pauker, O. & Hippe, H. (1997). Phylogenetic analysis of the genus Desulfotomaculum: evidence for the misclassification of *Desulfotomaculum guttoideum* and description of *Desulfotomaculum orientis* as *Desulfosporosinus orientis* gen. nov., comb. nov. *Int J Syst Evol Microbiol* **47**, 1134–1139.

Stackebrandt, E., Schumann, P., Schüler, E. & Hippe, H. (2003). Reclassification of *Desulfotomaculum auripigmentum* as *Desulfosporosinus auripigmenti* corrig., comb. nov. *Int J Syst Evol Microbiol* **53**, 1439–1443.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* **30**, 2725–2729.

Tebo, B. M. & Obraztsova, A. Y. (1998). Sulfate-reducing bacterium grows with Cr(VI), U(VI), Mn(IV), and Fe(III) as electron acceptors. *FEMS Microbiol Lett* **162**, 193–198.

Vaz-Moreira, I., Figueira, V., Lopes, A. R., Lobo-da-Cunha, A., Spröer, C., Schumann, P., Nunes, O. C. & Manaia, C. M. (2012). *Bacillus purgationiresistans* sp. nov., isolated from a drinking-water treatment plant. *Int J Syst Evol Microbiol* **62**, 71–77.

Werkman, C. H. & Weaver, H. J. (1927). Studies in the bacteriology of suphur stinker spoilage of canned sweet corn. *Iowa State Coll J Sci* **2**, 57–67.

Wielinga, B., Mizuba, M. M., Hansel, C. M. & Fendorf, S. (2001). Iron promoted reduction of chromate by dissimilatory iron-reducing bacteria. *Environ Sci Technol* **35**, 522–527.

Wilkins, M. J., Livens, F. R., Vaughan, D. J. & Lloyd, J. R. (2006). The Impact of Fe(III)-reducing bacteria on uranium mobility. *Biogeochemistry* **78**, 125–150.

Wu, C. Y., Zhuang, L., Zhou, S. G., Li, F. B. & Li, X. M. (2010). Fe(III)-enhanced anaerobic transformation of 2,4-dichlorophenoxyacetic acid by an iron-reducing bacterium *Comamonas koreensis* CY01. *FEMS Microbiol Ecol* **71**, 106–113.

Yang, G., Chen, J. & Zhou, S. (2015). *Novibacillus thermophilus* gen. nov., sp. nov., a gram-staining-negative and moderately thermophilic member of the family Thermoactinomycetaceae. *Int J Syst Evol Microbiol* **65**, 2591–2597.