Carboxydothermus islandicus sp. nov., a thermophilic, hydrogenogenic, carboxydotrophic bacterium isolated from a hot spring

Andrey A. Novikov,[1] Tatyana G. Sokolova,[2] Alexander V. Lebedinsky,[2] Tatyana V. Kolganova[3] and Elizaveta A. Bonch-Osmolovskaya[2]

Correspondence
Andrey A. Novikov
setchemist@gmail.com

[1]Gubkin Russian State University of Oil and Gas, Leninskiy Prospect 65, 117485 Moscow, Russia

[2]Winogradsky Institute of Microbiology, Russian Academy of Sciences, Prospect 60 Let Oktyabrya 7/2, 117312 Moscow, Russia

[3]Bioengineering Center, Russian Academy of Sciences, Prospect 60 Let Oktyabrya 7/1, 117312 Moscow, Russia

An anaerobic, thermophilic bacterium, strain SET IS-9^T, was isolated from an Icelandic hot spring. Cells of strain SET IS-9^T are short, slightly curved, motile rods. The strain grows chemolithotrophically on CO, producing equimolar quantities of H_2 and CO_2. It also grows fermentatively on lactate or pyruvate in the presence of yeast extract (0.2 g l^{-1}). Products of pyruvate fermentation are acetate, CO_2 and H_2. Growth occurs at 50–70 °C, with an optimum at 65 °C, and at pH 5.0–8.0, with an optimum at pH 5.5–6.0. The generation time during chemolithotrophic growth on CO under optimal conditions is 2.0 h. 16S rRNA gene sequence analysis suggested that the organism belongs to the genus *Carboxydothermus*. On the basis of phenotypic features and phylogenetic analysis, *Carboxydothermus islandicus* sp. nov. is proposed, with the type strain SET IS-9^T (=DSM 21830^T =VKM B-2561^T). An emended description of the genus *Carboxydothermus* is also given.

Microbial conversion of CO to H_2 by thermophiles can be accomplished by phylogenetically diverse prokaryotes (Sokolova *et al.*, 2009) and has been known since the isolation of *Carboxydothermus hydrogenoformans* (Svetlichny *et al.*, 1991). At the time of writing, the genus *Carboxydothermus* contains three species with validly published names: *C. hydrogenoformans* (Svetlichny *et al.*, 1991), *C. ferrireducens* (Slobodkin *et al.*, 2006) and *C. siderophilus* (Slepova *et al.*, 2009). Strain R1^T =DSM 7242^T, initially described as '*Carboxydothermus restrictus*' (Svetlichny *et al.*, 1994), was later shown to belong to a different phylogenetic lineage and was reclassified as *Thermolithobacter carboxydivorans* (Sokolova *et al.*, 2007). Here, we describe a novel species of the genus *Carboxydothermus*.

Strain SET IS-9^T was isolated from a sample of water, mud and grey filaments from an Icelandic hot spring with a temperature of 68.6 °C and a pH of 6.5 in the Hveragerdi area (64° 0′ 53″ N, 21° 11′ 18″ W). For the enrichment and isolation of this strain, medium 1 was used (l^{-1}): 0.66 g NH_4Cl, 0.16 g $MgCl_2.6H_2O$, 0.1 g $CaCl_2.6H_2O$, 0.33 g KCl, 0.5 g KH_2PO_4, 1 ml trace element solution (Kevbrin & Zavarzin, 1992) and 1 ml vitamin solution (Wolin *et al.*, 1963). After boiling, the medium was flushed with N_2 and cooled, $NaHCO_3$ (0.5 g l^{-1}) and $Na_2S.9H_2O$ (1.0 g l^{-1}) were added, and the pH was adjusted to 6.8–7.0 with 6 M HCl. Medium 1 was dispensed into 55 ml bottles in 10 ml portions. Bottles were inoculated with approximately 1 ml sample per bottle under an N_2 flow, then the gas phase was changed to 100 % CO at ambient pressure. The bottles were incubated at 65 °C. After 5 days incubation, the pressure in the inoculated bottles increased to 130–140 kPa, the CO content in the gas phase decreased and H_2 appeared. The concentrations of CO, H_2, CO_2 and volatile fatty acids were determined by using GC, performed as described previously (Sokolova *et al.*, 2002). A pure culture was obtained by using sequential serial dilutions and isolation of single colonies. Single colonies were obtained in medium 1 solidified with 5 % agar in roll tubes with 100 % CO in the gas phase.

Light microscopy, performed as described previously (Sokolova *et al.*, 2002), revealed the growth of rod-shaped cells. Colonies were brownish-yellow and 0.5 mm in diameter on the ninth day after inoculation. Electron

Abbreviations: AQDS, 9,10-anthraquinone-2,6-disulfonate; CFA, cellular fatty acid; DMA, dimethylacetal.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain SET IS-9^T is GQ324698.

Three supplementary figures are available with the online version of this paper.

microscopy (negative staining of whole cells with 1 % uranyl acetate; JEOL 100B electron microscope) revealed that cells were 0.5–1.0 μm wide and 1.5–2.5 μm long and had peritrichous flagella (Supplementary Fig. S1, available in IJSEM Online). The KOH test (Gregersen, 1978) was negative and cells stained Gram-positive, allowing us to suggest a Gram-positive cell-wall structure of strain SET IS-9^T.

The effects of temperature and pH on growth were studied in the same medium as was used for culture isolation. Growth was estimated by direct cell count. Growth of strain SET IS-9^T occurred within a temperature range of 50–70 °C, with an optimum at 65 °C, and at pH 5.0–8.0, with an optimum at pH 5.5–6.0. No growth was observed at 45 or 75 °C, or at pH 4.5 or 8.5. Strain SET IS-9^T grew chemolithotrophically on 100 % CO, producing H_2 and CO_2 according to the equation $CO + H_2O \rightarrow CO_2 + H_2$ (Fig. 1). No other products were detected. The generation time under optimal conditions on 100 % CO was 2.0 h. Growth of the new isolate on various substrates was tested in medium 1 at optimal pH and temperature values under 100 % N_2 in the gas phase in the case of non-gaseous substrates. Growth on $H_2 + CO_2$ (80 % H_2) or on the following substrates (2 g l^{-1}) was tested: pyruvate, glucose, cellobiose, sucrose, galactose, lactose, fructose, starch, glycerol, formate, acetate, peptone, yeast extract, lactate, ethanol and methanol, in both the presence and the absence of yeast extract (0.2 g l^{-1}). After three sequential transfers, growth was observed only in medium containing yeast extract (0.2 g l^{-1}) with lactate or pyruvate. During growth on pyruvate, the isolate produced acetate, CO_2 and H_2. Growth with various electron acceptors was tested in medium 1 containing yeast extract (0.2 g l^{-1}). In the case of Fe(III), 9,10-anthraquinone-2,6-disulfonate disodium salt (AQDS), NO_3^- or $S_2O_3^{2-}$, medium 1 devoid of Na_2S was used. Possible electron acceptors were added to a concentration of 2 g l^{-1}; S^0 was added to a concentration of 10 g l^{-1}. The following electron donors were tested: CO (100 %), H_2 (80 % $H_2 + CO_2$), acetate, lactate and pyruvate (2 g l^{-1}). Reduction of AQDS was determined from coloration change. Reduction of S^0, $S_2O_3^{2-}$ and SO_4^{2-} was determined by S^{2-} measurements (Trüper & Schlegel, 1964). Reduction of NO_3^- was determined with NO_2^-/NO_3^- Merck test paper. Among the electron acceptors tested, only AQDS was reduced in the presence of CO, acetate, lactate or pyruvate; however, growth was observed only in the presence of pyruvate, and it was not stimulated by AQDS. Penicillin, ampicillin, oxacillin or streptomycin (100 mg l^{-1}) inhibited growth on CO and production of H_2 completely.

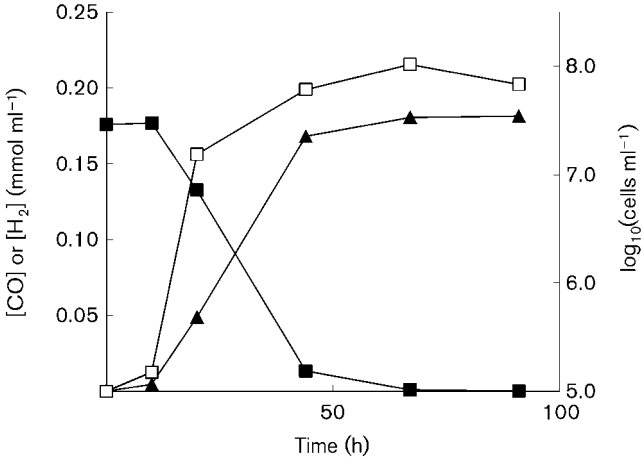

Fig. 1. Growth of strain SET IS-9^T in medium 1 under an atmosphere of 100 % CO at 65 °C. □, Cell number; ▲, H_2 production; ■, CO consumption. CO and H_2 are shown as their quantities in the gas phase [(ml liquid culture)$^{-1}$].

To determine the phylogenetic position of strain SET IS-9^T, DNA from its cells was isolated by a method based on a modified alkaline extraction procedure (Birnboim & Doly, 1979) and Promega Wizard technology. The G+C content of DNA was 37.7 ± 1.0 mol%, as determined by the thermal-denaturation method (Owen *et al.*, 1969). The 16S rRNA gene was amplified by PCR using the primers Bact 27f (5′-GTTTGATCMTGGCTCAG79-3′) and Univ 1492r (5′-TACGGYTACCTTGTTACGACTT-3′) (Lane, 1991). The PCR products were purified from low-melting-temperature agarose by using a Wizard PCR-Prep kit (Promega) according to the manufacturer's instructions. Sequencing was performed by using primers recommended by Lane (1991) and a BigDye Terminator v. 3.1 sequencing reaction kit on an ABI 3730 DNA automatic sequencer (Applied Biosystems). The 16S rRNA gene sequence of strain SET IS-9^T (1447 nt, corresponding to *Escherichia coli* positions 27–1450) has been deposited in GenBank under accession no. GQ324698. Preliminary phylogenetic analysis of the newly determined sequences was done with the NCBI BLAST server (Altschul *et al.*, 1997; http://blast.ncbi.nlm.nih.gov/Blast.cgi). The 16S rRNA gene sequence similarity values with the closest relatives revealed by BLAST were redetermined using the EzTaxon server 2.1 (Chun *et al.*, 2007; http://www.eztaxon.org/) in cases where BLAST failed to compare sequences along the entire lengths of their overlaps. Both BLAST and EzTaxon values were then recalculated so as to obtain similarity values with and without taking into account insertions/deletions; distinctions due to uncertainties (Ns etc.) were disregarded in both cases.

The close relatives of strain SET IS-9^T were determined to be the three currently recognized *Carboxydothermus* species. *C. siderophilus* exhibited 94.3 % 16S rRNA gene sequence similarity to strain SET IS-9^T if insertions/deletions were taken into account and 97.3 % if they were disregarded. The corresponding values for *C. hydrogenoformans* ranged, for the four gene copies present in its completely sequenced genome (Wu *et al.*, 2005), from 96.6 to 97.0 % and from 97.5 to 97.9 %, respectively. The values for the type strain of *C. ferrireducens* were 95.5 and 96.5 %, respectively. In all cases, the sizes and positions of the

fragments compared coincided with those of the sequenced fragment of the 16S rRNA gene of strain SET IS-9^T. Thus, if insertions/deletions were taken into account, the similarity values were not above the value (97 %) set by Tindall *et al.* (2010) as the threshold above which DNA–DNA hybridization is mandatory to claim species-level genotypic distinction. If insertions/deletions were disregarded, the similarity values were still not above the value of 98.7 %, noted by Stackebrandt & Ebers (2006) to make DNA–DNA hybridization unnecessary. The sequence similarity of the 16S rRNA gene of strain SET IS-9^T to corresponding genes of representatives of genera other than *Carboxydothermus* was low (the closest were *Moorella* spp. strains, exhibiting no more than 87 and 89 % 16S rRNA gene sequence similarity as calculated with and without taking into account insertions/deletions). Phylogenetic trees constructed by the neighbour-joining (Fig. 2) and maximum-parsimony (not shown) methods implemented in MEGA version 4 (Tamura *et al.*, 2007) after alignment (Supplementary Fig. S2, available in IJSEM Online) with built-in CLUSTAL W exhibited the same topology and confirmed the distinct phylogenetic position of strain SET IS-9^T within the genus *Carboxydothermus*. Therefore, we conclude that the phylogenetic position of strain SET IS-9^T corresponds to the status of a novel species in the genus *Carboxydothermus*.

The affiliation of strain SET IS-9^T to a novel species of the genus *Carboxydothermus* is supported by its phenotypic features (Table 1). Strain SET IS-9^T resembles other species of the genus *Carboxydothermus* in its cell morphology, the ability to grow lithotrophically on 100 % CO, and pH and temperature growth ranges. However, significant differences in metabolic patterns were observed. As distinct from other *Carboxydothermus* species, strain SET IS-9^T was unable to use any electron acceptor tested (AQDS, if reduced, did not stimulate growth or even inhibited it). In contrast to *C. hydrogenoformans* and *C. ferrireducens*, strain SET IS-9^T did not utilize H_2 and lactate as electron donors. In contrast to *C. siderophilus*, strain SET IS-9^T grew on pyruvate without any acceptors.

To obtain additional chemotaxonomic information, cellular fatty acid (CFA) profiles were determined for strain SET IS-9^T and *Carboxydothermus* species by GC-MS. For CFA analysis, cells were centrifuged at 3300 ***g*** for 15 min, resuspended in 4 ml H_2O and centrifuged again at 12 000 ***g*** for 15 min, and the cell pellet (approx. 50 mg) was frozen and then processed according to instructions for the Microbial Identification system (Sasser, 1990). The obtained extract of CFA methyl esters was then filtered, and a 1 μl sample was injected into the ThermoScientific Trace GC Ultra DSQ II GC-MS system (column TR-5MS 15 m × 0.25 mm; helium flow 1.2 ml min^{-1}; inlet 280 °C with split flow 20 ml min^{-1}; oven temperature programmed as 5 min at 120 °C, 10 °C min^{-1} to 250 °C, 3 min at 250 °C, 20 °C min^{-1} to 300 °C, 1.5 min at 300 °C; transfer line 300 °C; mass-selective detector in EI 70 eV mode). CFA content was determined as the percentage of the total ion current peak area. The predominant fatty acid species detected were $C_{14:0}$ (14.3 %), iso-$C_{15:0}$ (20.7 %) and $C_{16:0}$ (41.9 %); significant amounts of dimethylacetals (DMAs) and a low but detectable amount of nonadecane were also detected (Table 2). Polar lipids were extracted and analysed by two-dimensional TLC according to Tindall (1990). The polar lipid pattern was fairly simple, with only one predominant phospholipid in all species tested and minor amounts of other polar lipids, barely detectable with phosphomolybdic acid only (Supplementary Fig. S3, available in IJSEM Online). Thus, strain SET IS-9^T has a CFA profile different from those of known *Carboxydothermus* species: it exhibits a higher content of $C_{14:0}$ and

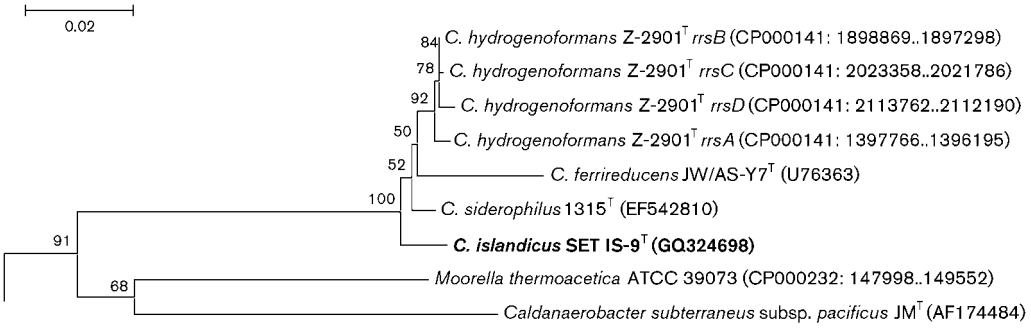

Fig. 2. 16S rRNA gene-based neighbour-joining dendrogram showing the relationships between strain SET IS-9^T and *Carboxydothermus* species. The dendrogram is a subtree of a tree constructed by using 16S rRNA gene sequences of the type strains of *Carboxydothermus* species, *Moorella thermoacetica* ATCC 39073, taken as the closest outgroup relative revealed by BLASTN, and type strains of *Firmicutes* species capable of hydrogenogenic carboxydotrophy and representing five families (the latter strains are not shown in the figure, except for *Caldanaerobacter subterraneus* subsp. *pacificus*). *C. hydrogenoformans* Z-2901^T *rrsA*, *B*, *C* and *D* are the four gene copies present in the completely sequenced genome. The tree was constructed by using the neighbour-joining method implemented in MEGA version 4 at default values of all parameters. There were 1333 positions in the final dataset. Bootstrap percentages (based on 500 replicates) are given at branching points. Bar, 0.02 base substitutions per site.

Table 1. Characteristics useful for differentiating *Carboxydothermus* species and strain SET IS-9^T

Taxa: 1, *C. hydrogenoformans* Z-2901^T; 2, *C. ferrireducens* JW/AS-Y7^T; 3, *C. siderophilus* 1315^T; 4, strain SET IS-9^T. Data on CFA profiles and substrate utilization were obtained in this work; other data for *C. hydrogenoformans* Z-2901^T are from Svetlichny *et al.* (1991), for *C. ferrireducens* JW/AS-Y7^T from Slobodkin *et al.* (1997, 2006) and for *C. siderophilus* 1315^T from Slepova *et al.* (2009). All taxa are unable to utilize lactate + sulfate. ND, Not determined.

Characteristic	1	2	3	4
Morphology	Slightly curved rods	Straight to slightly curved rods	Straight rods	Straight to slightly curved rods
Flagellation	Lateral flagella	Peritrichous flagella	Non-motile	Peritrichous flagella
Growth temperature range (optimum) (°C)	40–78 (70–72)	50–74 (65)	52–70 (65)	50–70 (65)
Growth pH range (optimum)	6.6–8.0 (7.0)	5.5–7.6 (6.0–6.2)	5.5–8.5 (6.5–7.2)	5.5–8.0 (5.5–6.0)
Generation time under optimal conditions (h)	2.0	ND	9.3	2.0
DNA G+C content (mol%)	39.0±1.0	41	41.5±0.5	37.7±1.0
Major fatty acids (% of total)				
$C_{14:0}$	11.3	7.4	ND	14.3 (25.6)*
$C_{15:0}$ iso	30.1	30.8	ND	20.7 (12.4)
$C_{15:0}$ anteiso	9.8	8.6	ND	1.1 (4.1)
$C_{16:0}$	20.2	24.4	ND	41.9 (26.2)
DMA total	5.5	15.4	ND	6.8 (25.7)
Substrate utilization				
100 % CO without yeast extract	+	−	−	+
100 % CO	+	−	−	+
Pyruvate	+	+	−	+
Lactate	−	+	−	+
CO + Fe(III)	−	+	+	−
H_2 + Fe(III)	+	+	−	−
CO + AQDS	+	+	+	−
H_2 + AQDS	+	+	−	−
Acetate + AQDS	−	−	−	−
Lactate + AQDS	+	+	+	−
Lactate + $S_2O_3^{2-}$	+	+	−	−
Lactate + S^0	+	+	−	−
Lactate + NO_3^-	+	+	−	−

*Fatty acid data for strain SET IS-9^T are given for cells grown on CO and cells grown on pyruvate (in parentheses).

$C_{16:0}$ and a lower content of iso-$C_{15:0}$ and anteiso-$C_{15:0}$ (differences in DMA content between *Carboxydothermus* species should be treated with caution, since, as seen from Table 2, DMA content in strain SET IS-9^T depended strongly on growth conditions).

To conclude, based on phylogenetic evidence and phenotypic features, we propose that strain SET IS-9^T should be assigned to a novel species of the genus *Carboxydothermus* with the name *Carboxydothermus islandicus* sp. nov.

Emended description of the genus *Carboxydothermus* Slobodkin *et al.* 2006

Rod-shaped, Gram-positive bacteria. Anaerobic and thermophilic. Neutrophilic. CO is utilized either with or without production of H_2. Some species are able to couple the reduction of an external electron acceptor such as Fe(III), SO_3^{2-}, $S_2O_3^{2-}$, S^0, NO_3^-, fumarate or AQDS with the oxidation of organic acids, polyols and H_2. In the presence as well as the absence of electron acceptor, organic substrates are oxidized incompletely to acetate as the main metabolic product. Sulfate is not reduced.

Description of *Carboxydothermus islandicus* sp. nov.

Carboxydothermus islandicus (is.lan′di.cus. N.L. masc. adj. *islandicus* pertaining to Iceland, Icelandic).

Cells are Gram-positive, motile, straight to slightly curved rods, 0.5–1.0 μm wide and 1.5–2.5 μm long with rounded ends. Colonies are brownish-yellow and 0.5 mm in diameter. Cells stain Gram-positive, occur singly or in pairs and exhibit peritrichous flagella. Spores are not observed. Strictly anaerobic. Growth occurs at 50–70 °C, with an optimum at 65 °C, and at pH 5.0–8.0, with an optimum at pH 5.5–6.0. Grows chemolithoautotrophically and hydrogenogenically on CO; grows fermentatively on lactate or pyruvate under N_2 in the presence of yeast extract. Products of pyruvate fermentation are acetate, CO_2 and H_2. Does not grow on $H_2 + CO_2$ (8 : 2) in the presence

Table 2. Cellular fatty acid contents (%) of strain SET IS-9^T and known *Carboxydothermus* species

Taxa: 1, *C. hydrogenoformans* Z-2901^T; 2, *C. ferrireducens* JW/AS-Y7^T; 3, strain SET IS-9^T grown on CO; 4, strain SET IS-9^T grown on pyruvate. Because of the absence of a common nutrient combination supporting good growth of all of the strains studied, for CFA analysis *C. hydrogenoformans* Z-2901^T was grown on medium 1 supplemented with 0.2 g yeast extract l^{-1} under 100 % CO for 72 h, *C. ferrireducens* JW/AS-Y7^T was grown on medium 1 supplemented with 20 mM sodium fumarate, 20 mM glycerol and 0.2 g yeast extract l^{-1} under 100 % N_2 for 48h, and strain SET IS-9^T was grown either on CO as for *C. hydrogenoformans* Z-2901^T or on medium 1 supplemented with 2.0 g sodium pyruvate l^{-1} and 0.2 g yeast extract l^{-1} under 100 % N_2 for 72 h. ND, Not detected; tr, <1.0 %.

Fatty acid	1	2	3	4
$C_{12:0}$ iso	6.7	ND	1.0	1.0
$C_{14:0}$	11.3	7.4	14.3	25.6
$C_{15:0}$ iso	30.1	30.8	20.7	12.4
$C_{15:0}$ anteiso	9.8	8.6	1.1	4.1
$C_{14:0}$ DMA	tr	1.4	tr	1.8
$C_{15:0}$	2.3	1.9	4.5	1.0
$C_{15:0}$ iso DMA	tr	1.0	tr	1.6
$C_{16:0}$ amine	tr	1.2	tr	tr
$C_{16:0}$ iso	3.9	2.4	3.6	tr
$C_{16:0}$	20.2	24.4	41.9	26.2
$C_{16:0}$ iso DMA	3.7	9.3	5.8	21.8
$C_{17:0}$ iso	2.8	1.5	1.5	tr
$C_{17:0}$ anteiso	3.2	tr	tr	ND
$C_{18:0}$ DMA 1*	tr	1.8	tr	tr
$C_{18:0}$ DMA 2*	ND	1.9	tr	tr
$C_{18:0}$	3.0	1.0	2.0	1.8
$C_{19:0}$ alkane	tr	tr	tr	1.2

*$C_{18:0}$ DMA 1 and $C_{18:0}$ DMA 2 are $C_{18:0}$ DMAs differing in their structure (straight-chain or branched), which could not be determined from the mass spectra obtained.

or absence of Fe(III), AQDS, SO_4^{2-}, S^0, NO_3^- or $S_2O_3^{2-}$. Does not reduce Fe(III), S^0, NO_3^- or $S_2O_3^{2-}$ with CO, H_2, acetate, lactate or pyruvate. Does not ferment glucose, cellobiose, sucrose, galactose, lactose, fructose, starch, glycerol, formate, acetate, peptone, lactate, ethanol or methanol, in either the presence or the absence of yeast extract. Growth is inhibited completely by penicillin, ampicillin, oxacillin and streptomycin. Major CFAs are $C_{14:0}$, iso-$C_{15:0}$ and $C_{16:0}$. The DNA G+C content of the type strain is 37.7±1.0 mol%.

The type strain, SET IS-9^T (=DSM 21830^T =VKM B-2561^T), was isolated from a hot spring in Iceland.

Acknowledgements

We are grateful to Anna Perevalova and Christa Schlepper for providing samples, to Lyubov Didenko for electron microscopy and to Ekaterina Detkova for DNA G+C content analysis. *C. ferrireducens* JW/AS-Y7^T was kindly provided by Sergey Gavrilov, Winogradsky Institute of Microbiology. This work was supported by the Federal Target Program 'Scientific and Scientific-Pedagogical Personnel of Innovative Russia 2009–2013' and the Russian Academy of Sciences 'Molecular and Cell Biology' and 'Origin and Evolution of the Biosphere' programmes.

References

Altschul, S. F., Madden, T. L., Schäffer, A. A., Zhang, J., Zhang, Z., Miller, W. & Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* **25**, 3389–3402.

Birnboim, H. C. & Doly, J. (1979). A rapid alkaline extraction procedure for screening recombinant plasmid DNA. *Nucleic Acids Res* **7**, 1513–1523.

Chun, J., Lee, J.-H., Jung, Y., Kim, M., Kim, S., Kim, B. K. & Lim, Y. W. (2007). EzTaxon: a web-based tool for the identification of prokaryotes based on 16S ribosomal RNA gene sequences. *Int J Syst Evol Microbiol* **57**, 2259–2261.

Gregersen, T. (1978). Rapid method for distinction of Gram-negative from Gram-positive bacteria. *Eur J Appl Microbiol Biotechnol* **5**, 123–127.

Kevbrin, V. V. & Zavarzin, G. A. (1992). Effect of sulfur compounds on the growth of the halophilic homoacetic bacterium *Acetohalobium arabaticum*. *Mikrobiologiia* **61**, 812–817.

Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester, UK: Wiley.

Owen, R. J., Hill, L. R. & Lapage, S. P. (1969). Determination of DNA base compositions from melting profiles in dilute buffers. *Biopolymers* **7**, 503–516.

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

Slepova, T. V., Sokolova, T. G., Kolganova, T. V., Tourova, T. P. & Bonch-Osmolovskaya, E. A. (2009). *Carboxydothermus siderophilus* sp. nov., a thermophilic, hydrogenogenic, carboxydotrophic, dissimilatory Fe(III)-reducing bacterium from a Kamchatka hot spring. *Int J Syst Evol Microbiol* **59**, 213–217.

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.

Slobodkin, A. I., Sokolova, T. G., Lysenko, A. M. & Wiegel, J. (2006). Reclassification of *Thermoterrabacterium ferrireducens* as *Carboxydothermus ferrireducens* comb. nov., and emended description of the genus *Carboxydothermus*. *Int J Syst Evol Microbiol* **56**, 2349–2351.

Sokolova, T. G., Kostrikina, N. A., Chernyh, N. A., Tourova, T. P., Kolganova, T. V. & Bonch-Osmolovskaya, E. A. (2002). *Carboxydocella thermautotrophica* gen. nov., sp. nov., a novel anaerobic, CO-utilizing thermophile from a Kamchatkan hot spring. *Int J Syst Evol Microbiol* **52**, 1961–1967.

Sokolova, T. G., Hanel, J., Onyenwoke, R. U., Reysenbach, A.-L., Banta, A., Geyer, R., González, J. M., Whitman, W. B. & Wiegel, J. (2007). Novel chemolithotrophic, thermophilic, anaerobic bacteria *Thermolithobacter ferrireducens* gen. nov., sp. nov. and *Thermolithobacter carboxydivorans* sp. nov. *Extremophiles* **11**, 145–157.

Sokolova, T. G., Henstra, A. M., Sipma, J., Parshina, S. N., Stams, A. J. M. & Lebedinsky, A. V. (2009). Diversity and ecophysiological features of thermophilic carboxydotrophic anaerobes. *FEMS Microbiol Ecol* **68**, 131–141.

Stackebrandt, E. & Ebers, J. (2006). Taxonomic parameters revisited: tarnished gold standards. *Microbiol Today* **33**, 152–155. http://www.sgm.ac.uk/pubs/micro_today/pdf/110602.pdf

Svetlichny, V. A., Sokolova, T. G., Gerhardt, M., Ringpfeil, M., Kostrikina, N. A. & Zavarzin, G. A. (1991). *Carboxydothermus hydrogenoformans* gen. nov., sp. nov., a CO-utilizing thermophilic anaerobic bacterium from hydrothermal environments of Kunashir Island. *Syst Appl Microbiol* **14**, 254–260.

Svetlichny, V. A., Sokolova, T. G., Kostrikina, N. A. & Lysenko, A. M. (1994). *Carboxydothermus restrictus* sp. nov. – a new thermophilic anaerobic carboxydotrophic bacterium. *Mikrobiologiya* **63**, 523–528 (in Russian).

Tamura, K., Dudley, J., Nei, M. & Kumar, S. (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* **24**, 1596–1599.

Tindall, B. J. (1990). Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett* **66**, 199–202.

Tindall, B. J., Rosselló-Móra, R., Busse, H.-J., Ludwig, W. & Kämpfer, P. (2010). Notes on the characterization of prokaryote strains for taxonomic purposes. *Int J Syst Evol Microbiol* **60**, 249–266.

Trüper, H. G. & Schlegel, H. G. (1964). Sulfur metabolism in *Thiorhodaceae*. I. Quantitative measurements on growing cells of *Chromatium okenii*. *Antonie van Leeuwenhoek* **30**, 225–238.

Wolin, E. A., Wolin, M. J. & Wolfe, R. S. (1963). Formation of methane by bacterial extracts. *J Biol Chem* **238**, 2882–2886.

Wu, M., Ren, Q., Durkin, A. S., Daugherty, S. C., Brinkac, L. M., Dodson, R. J., Madupu, R., Sullivan, S. A., Kolonay, J. F. & other authors (2005). Life in hot carbon monoxide: the complete genome sequence of *Carboxydothermus hydrogenoformans* Z-2901. *PLoS Genet* **1**, e65.