***Desulfacinum hydrothermale* sp. nov., a thermophilic, sulfate-reducing bacterium from geothermally heated sediments near Milos Island (Greece)**

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A thermophilic, sulfate-reducing bacterium, strain MT-96^T, was isolated from an active, marine, shallow-water hydrothermal vent system. It used a large variety of substrates, ranging from simple organic compounds to long-chain fatty acids, as electron donors. Autotrophic growth was possible with H₂ and CO₂ in the presence of sulfate. Sulfate, thiosulfate and sulfite were used as electron acceptors. Sulfur and nitrate were not reduced. Fermentative growth was obtained with pyruvate, but not with fumarate or malate. Substrate oxidation was usually complete, leading to production of CO₂, but at high substrate concentrations acetate accumulated. The oval-shaped cells were 0.8–1.0 µm in width and 1.5–2.5 µm in length. Cells were motile during the early-exponential-growth phase, but motility rapidly declined during later growth phases. Spores were not produced and cells stained Gram-negative. The temperature limits for growth were between 37 and 64 °C, with an optimum at 60 °C. Growth was observed at salinities ranging from 15 to 78 g NaCl l⁻¹, with optimum growth in the presence of 32–36 g NaCl l⁻¹. This might reflect an adaptation to the elevated salinity of the hydrothermal fluid. The G+C content of the DNA was 59.5 mol%. Vitamins or other supplements were not required. Based on the 16S rRNA gene sequence, strain MT-96^T belonged in the δ-subclass of the *Proteobacteria*. Strain MT-96^T was found to be phenotypically and phylogenetically related to *Desulfacinum infernum* (<95.3% sequence identity) and represents a new member of the genus *Desulfacinum*. The name *Desulfacinum hydrothermale* is proposed for this strain; the type strain is MT-96^T (= DSM 13146).

Keywords: *Desulfacinum*, thermophilic sulfate-reducing bacterium, hydrothermal vent, deep subsurface biosphere

INTRODUCTION

Most thermophilic, dissimilatory, sulfate-reducing bacteria are phylogenetically members of the *Clostridium*–*Bacillus* subphylum of the Gram-positive bacteria (Stackebrandt *et al.*, 1997) or a deeply branching lineage within the domain *Bacteria* containing the genera *Thermodesulfobacterium* and *Thermodesulfovibrio* (Zeikus *et al.*, 1983; Henry *et al.*,

1994). Within the δ-subclass of the *Proteobacteria*, which contains all other sulfate-reducing bacteria, only three thermophilic species are described. These are *Desulfacinum infernum* (Rees *et al.*, 1995), *Thermodesulforhabdus norvegicus* (Beeder *et al.*, 1995) and the incompletely described, alkane-oxidizing strain TD3 (Rueter *et al.*, 1994); all were isolated from marine habitats and require marine or brackish water media for cultivation.

Two different habitats could be considered as ideal sites for marine thermophilic sulfate reducers. These are oil reservoirs located several kilometres below the sea floor, which have elevated temperatures and pressures (e.g. Stetter *et al.*, 1993; Nilsen *et al.*, 1996b),

Abbreviation: MPN, most probable number.

The GenBank accession number for the nearly complete 16S rRNA sequences of strains MT-96^T and M40/2 CIV-2.3, and the partial sequence of strain M40/2 CIV-3.2 are AF170417, AF170418 and AF170420, respectively.

and hydrothermal vent systems (Jørgensen *et al.*, 1990, 1992). In addition, a common deep subterranean biosphere might exist that supports the growth of these organisms as has been suggested by Gold (1992). Recently, data were presented that support such an idea (e.g. Deming & Baross, 1993; L'Haridon *et al.*, 1995; Nilsen *et al.*, 1996b; Summit & Baross, 1998; but see Stetter *et al.*, 1993 for a different view). For example, the thermophilic, marine sulfate reducer *Desulfacinum infernum* and *Thermodesulforhabdus norvegicus* were isolated from enrichment cultures inoculated with formation water from North Sea oilfields (Rees *et al.*, 1995; Beeder *et al.*, 1995). A detailed study of the distribution of thermophilic, marine sulfate reducers in North Sea oilfield waters and oil reservoirs by using genus-specific, fluorescent antibodies showed a clear dominance of the genera *Archaeoglobus* and *Thermodesulforhabdus*, whereas thermophilic *Desulfotomaculum* spp. were only obtained from enrichment cultures (Nilsen *et al.*, 1996a, b). In addition, several halophilic or halo-tolerant *Desulfotomaculum* spp. were isolated from various geothermally influenced, terrestrial subsurface habitats (Daumas *et al.*, 1988; Nazina *et al.*, 1989; Love *et al.*, 1993; Fardeau *et al.*, 1995; Liu *et al.*, 1997; Tardy-Jacquenod *et al.*, 1998).

The increased interest in thermophilic, sulfate-reducing bacteria has been strongly connected to their role and function during oil recovery, i.e. an enhanced sulfide production and stimulated corrosion due to the activities of sulfate-reducing bacteria. Little is known about the natural role of thermophilic sulfate reducers. Since the isolation of the hydrocarbon-degrading strain TD3 from a deep-sea vent site in the Guaymas Basin, the substrate spectra of thermophilic, sulfate-reducing bacteria has been extended and now includes alkanes, which were originally thought to be recalcitrant under anoxic conditions (Rueter *et al.*, 1994). In this paper we describe a thermophilic isolate, MT-96^T, which was obtained from a shallow, submarine hydrothermal vent. The data presented in this communication further suggest that members of the thermophilic genera *Thermodesulforhabdus* and *Desulfacinum* are, indeed, part of a subsurface microbial community, as originally proposed by Nilsen *et al.* (1996b).

METHODS

Source of organism. Strain MT-96^T was isolated from most probable number (MPN) dilution series that were performed to investigate the horizontal and vertical distribution of sulfate-reducing bacteria at a shallow, submarine hydrothermal vent located in Palaeochori Bay, which is a sandy bay in the south-eastern part of the Greek island of Milos in the Aegean Sea (Sievert *et al.*, 1999).

Media and culture conditions. The MPN series was performed as described by Sievert *et al.* (1999). Artificial, bicarbonate-buffered seawater medium, as defined for sulfate-reducing bacteria, was used (Widdel & Bak, 1992); 10 mM acetate as carbon source, non-chelated trace element mixture number 1, vitamins, and a tungstate and selenite

solution were added (Widdel & Bak, 1992). For isolation and routine cultivation, acetate was replaced by lactate. Media were reduced with sulfide and prepared as described by Widdel & Bak (1992). Pure cultures were obtained by repeated use of deep agar dilution series (Widdel & Bak, 1992). Substrate utilization was determined by adding the carbon and energy sources from sterile stock solutions, and the preparations were incubated for about 3 weeks. To test the capability for autotrophic growth, cultures were grown with a headspace of 80% H₂/20% CO₂ at an overpressure of 101.29 kPa. The temperature range for growth was determined by incubation in a temperature gradient block from 35 to 80 °C in increments of 2–4 °C. The pH range for growth was determined in mineral medium with pH from 5.0 to 9.0. The dependence of growth on the concentration of NaCl was determined in mineral medium with NaCl concentrations from 0 to 82 g NaCl l⁻¹.

Chemical and biochemical characterization. The presence of desulfoviridin was tested as described by Postgate (1956). The G+C content was determined by M. Nagel (Fachhochschule Bremerhaven, Germany) by HPLC as described previously (Brinkhoff *et al.*, 1999). Lambda DNA was used for final correction of the values.

PCR amplification and sequencing of the 16S rRNA gene. To amplify the almost complete 16S rRNA encoding gene (1500 bp) of strains MT-96^T, M40/2 CIV-2.3 and M40/2 CIV-3.2, primers GM3F and GM4R were used in a 35 cycle PCR with an annealing temperature of 40 °C (Muyzer *et al.*, 1995). PCR products were purified by using the QIAquick Spin PCR Purification kit (Qiagen) as described by the manufacturer. The *Taq* DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems) was used to directly sequence the PCR products according to the protocol provided by the manufacturer. The sequencing primers were described by Buchholz-Cleven *et al.* (1997). The sequence reaction mixtures were electrophoresed on an Applied Biosystems 373S DNA sequencer.

Phylogenetic analyses of 16S rRNA gene sequence data. The sequences were loaded into the 16S rRNA sequence database of the Technical University Munich using the program package ARB (O. Strunk and others, <http://www.mikro.biologie.tu-muenchen.de>). The tool ARB-ALIGN was used for sequence alignment. The alignment was visually inspected and corrected manually. Tree topologies were evaluated by performing maximum-parsimony, neighbour-joining and maximum-likelihood analyses. Only sequences with at least 1400 nucleotides were used for the calculation of different trees. The partial sequence of strain M40/2 CIV-3.2 (1268 nucleotides) was added to the reconstructed tree by applying parsimony criteria without allowing changes in the overall tree topology. The strain designations and nucleotide sequence accession numbers are as follows: *Desulfacinum infernum* ACM 3991^T, L27426; *Thermodesulforhabdus norvegicus* DSM 9990^T, U25627; *Desulforhabdus amnigenus* DSM 10338^T, X83274; strain TD3, X80922; *Desulfohalobium retbaense* DSM 5692^T, U48244; *Desulforhopalus vacuolatus* DSM 9700^T, L42613; *Desulfobacter postgatei* DSM 2034^T; M26633; '*Desulfobacterium niacini*' DSM 2650^T, U51845; *Desulfosarcina variabilis* DSM 2060^T, M34407; *Syntrophus buswellii* DSM 2612^T, X85131; *Desulfomonile tiedjei* ATCC 49306^T M26635; *Syntrophobacter wolinii* DSM 2805^T, X70905; *Desulfuromonas acetoxidans* ATCC 11775^T, X80725; *Desulfovibrio halophilus* DSM 5663^T, U48243; *Desulfovibrio desulfuricans* ATCC

27774, M34113; *Desulfovibrio gabonensis* DSM 10636^T, U31080. The sequence accession number of clone OPB33 is AF026987.

RESULTS

Enrichment and isolation

Serial MPN dilutions were performed using media containing 10 mM acetate as the only carbon source and inocula from different layers of the sediment (Sievert *et al.*, 1999). Strain MT-96^T was isolated from the 10⁻² dilution of a sample that came from the sediment layers between 2 and 3 cm at a distance of 10 cm from the vent centre (Sievert *et al.*, 1999). The *in situ* temperature of this sediment layer ranged from 50 to 60 °C and the pH was approximately 5.0 (Sievert *et al.*, 1999). The mean composition of the discharged gases from different seeps in Palaeochori Bay was 80.5% (v/v) CO₂, 1.2% (v/v) H₂S, 0.8% (v/v) CH₄ and 0.4% (v/v) H₂ (Dando *et al.*, 1995). The chemically reduced hydrothermal fluid had an elevated salinity of up to 58%, compared to 39% of the ambient seawater (Thiermann *et al.*, 1997). After 2–3 weeks incubation, formation of sulfide and growth of small, oval rods were observed. Following several transfers into liquid media, a pure culture was obtained by repeated agar dilution series using lactate as electron donor and sulfate as electron acceptor. Previous experiments had shown that lactate allows faster growth. Besides strain MT-96^T, which was isolated from a vent site at a water depth of 8 m, two other thermophilic, sulfate-reducing isolates were obtained from a vent site that was located offshore of the southeastern part of the island of Milos (Greece) at 120 m water depth using the same procedures as indicated above. These samples were obtained on a cruise with the R/V Meteor during leg M40/2. A MPN series with acetate as single carbon source inoculated with sediment from a horizon 45–65 mm below the surface (*in situ* temperature 60–70 °C) showed growth at the 10⁻² and 10⁻³ dilutions. Strains M40/2 CIV-2.3 and M40/2 CIV-3.2 were isolated from the 10⁻² and 10⁻³ dilutions, respectively. Further physiological characterization of these two strains was not carried out, but they were included in the phylogenetic analysis since they clustered with MT-96^T and *Thermodesulforhabdus norvegicus*. In contrast to the shallow-water hydrothermal vent, the surface layer of the deeper vent site was covered with large, non-motile, filamentous sulfur-oxidizing bacteria, which were attached to sediment particles. The *in situ* temperature at the surface was around 45 °C.

Cell morphology

Cells of strain MT-96^T grown with lactate or acetate and sulfate were oval or short rods measuring 0.8–1.0 × 1.5–2.5 µm (Fig. 1). Cells occurred singly or in pairs. Spore formation was absent. Cells were motile only during the early-exponential-growth phase. In

Fig. 1. Scanning electron micrograph of strain MT-96^T showing the typical oval or rod-shaped cell form. Bar, 1 µm.

later growth phases, motility was only occasionally observed. Very often, only a few cells were motile.

DNA base ratio

The G + C content of the DNA was 59.5 mol %.

Physiological properties

The optimum growth temperature of strain MT-96^T was determined to be 60 °C. No growth was observed at temperatures higher than 64 °C and lower than 37 °C. At temperatures less than 44 °C, cells aggregated into flocs. The pH range was between 6.0 and 7.5 with an optimum at pH 7.0. The optimum NaCl concentration for growth was between 32 and 36 g l⁻¹. Growth was obtained at salinities ranging from 15 to 78 g NaCl l⁻¹. Vitamins were not essential for growth (six consecutive transfers in vitamin-free medium). Desulfovibridin was absent in strain MT-96^T.

Strain MT-96^T used the following compounds as electron donors and carbon sources in the presence of 10 mM sulfate [added substrate concentrations (mM) are given in parentheses]: hydrogen plus carbon dioxide (80%/20%, v/v) (four consecutive transfers without organic substrate; in medium with N₂/CO₂ as headspace no growth was observed), formate (10), acetate (10), propionate (10), butyrate (5), isobutyrate (5), valerate (5), isovalerate (5), octanoate (2.5), decanoate (0.5), dodecanoate (0.5), hexadecanoate (1), octadecanoate (1), ethanol (10), propanol (10), butanol (10), hexanol (5), lactate (10), pyruvate (10), alanine (10), yeast extract (0.1% and 0.01%).

The following substrates were tested, but not utilized: fructose (5), glucose (5), sucrose (5), malate (10),

Fig. 2. Maximum-likelihood tree based on 1426 positions of nearly full length 16S rRNA sequences from 20 bacteria and one clonal sequence (OPB33) from the δ -subclass of the *Proteobacteria*, rooted with *Escherichia coli* (γ -subclass of the *Proteobacteria*). All trees constructed with other reconstruction algorithms (neighbour-joining and parsimony) resulted in the same overall topology. In bootstrap analysis using neighbour-joining and parsimony criteria, the branching leading to the *Desulfacinum*–*Thermodesulfobacter* cluster was supported with values of 100 (data not shown). The partial sequence of strain M40/2 CIV-3.2 was inserted into the tree by applying parsimony criteria without allowing for changes in the overall tree topology. Scale-bar represents 10 substitutions per 100 nucleotides. T indicates type strain; see Methods for strain and nucleotide sequence accession numbers.

fumarate (10), succinate (10), citrate (10), glycerol (10), glutamate (10), benzoate (2), *p*-cresol (1), hexadecane (1), crude oil (0.001 %).

Sulfate, sulfite and thiosulfate were used as electron acceptors. Sulfur and nitrate did not serve as electron acceptor. Pyruvate allowed slow growth by fermentation.

The minimum doubling time with lactate was 23 h. Increased substrate concentrations resulted in the production of acetate, which was released into the medium. When grown on 20 mM lactate, 2–3 mM acetate and 0.5 mM propionate were observed.

Phylogenetic analyses

The 16S rRNA gene sequence of strain MT-96^T shared between 80 and 95.3 % identity with the 16S rRNA gene sequences of other sulfate reducers of the δ -

subclass of the *Proteobacteria* (data not shown). The phylogenetic positions of strains MT-96^T, M40/2 CIV-2.3 and M40/2 CIV-3.2 are shown in Fig. 2. All of them are members of the δ -subclass of the *Proteobacteria*. Comparative phylogenetic analyses revealed that strain MT-96^T is a member of the genus *Desulfacinum* and is closely related to *Desulfacinum infernum* (95.3 % 16S rRNA sequence identity), which is the only species of this genus reported to date (Fig. 2). Strain M40/2 CIV-2.3 was closely related to MT-96^T (98.2 % 16S rRNA sequence identity), whereas strain M40/2 CIV-3.2 clustered with *Thermodesulfobacter norvegicus* (94.9 % 16S rRNA sequence identity).

DISCUSSION

The isolate strain MT-96^T is a thermophilic, sulfate-reducing bacterium that can use a large variety of

Table 1. Comparison of selected characteristics of strain MT-96^T, *Desulfacinum infernum*, *Thermodesulforhabdus norvegicus*, strain TD3 and *Desulforhabdus amnigenus*

All strains contained desulfovirdin and usually showed complete substrate oxidation. Data were obtained from Rueter *et al.* (1994); Beeder *et al.* (1995); Rees *et al.* (1995); Oude Elferink *et al.* (1995); and Ehrenreich (1996).

Character	Strain MT-96 ^T	<i>Desulfacinum infernum</i>	<i>Thermodesulforhabdus norvegicus</i>	Strain TD3	<i>Desulforhabdus amnigenus</i>
Morphology	Oval	Oval	Rod	Rod	Rod
Size (µm)	0.8–1.0 × 1.5–2.5	1.5 × 2.5–3.0	1.0 × 2.5	0.8–1.0 × 2.0–3.5	1.4–1.9 × 2.5–3.4
Motility	+*	—	+†	—/+	—
G + C content (mol %)	59.5	64.0	51.0	37.4	52.5
Optimal salinity (g l ⁻¹)	32–36	10	16	NR	0
Optimal temperature (°C)	60	60	60	60	37
Electron donors:					
H ₂ + CO ₂	+‡	+‡	—	—	+‡
Formate	+‡	+‡	—	—	+‡
Acetate	+	+	+	—	+
Fatty acids (no. C atoms)	3–5, 8, 10, 12, 16, 18	3–18	4–18	4–8, 12, 16, 18	3–4
Isobutyrate	+	+	NR	NR	+
Isovalerate	+	+	NR	NR	—
Ethanol	+	+	+	—	+
Lactate	+	+	+	—	+
Pyruvate	+	+	+	—	+
Fumarate	—	+	+	—	—
Succinate	—	+	+	—	—
Malate	—	+	+	—	—
Benzoate	—	—	—	—	—
Hexadecane	—	—	—	+	NR
Others	Propanol, butanol, hexanol, alanine	Propanol, butanol, hexanol, alanine	NR	Alkanes	Propanol, butanol
Fermentative growth on pyruvate	+	+	—	NR	—
Electron acceptors:					
Sulfate	+	+	+	+	+
Sulfite	+	+	+	NR	+
Sulfur	—	—	—	NR	—
Thiosulfate	+	+	—	—	+
Nitrate	—	—	—	NR	—
Growth factor requirement	None	Vitamins	None	NR	NR

NR, Not reported.

* Cells are motile during early-exponential-growth phase, but motility can rapidly decline during growth.

† Single polar flagellum.

‡ Autotrophic growth.

substrates as electron donors. The ability to use acetate and propionate and to grow autotrophically with H₂ and CO₂ is shared with its closest relative, *Desulfacinum infernum* (Rees *et al.*, 1995) and several mesophilic sulfate reducers (Widdel & Bak, 1992). The substrates are usually completely oxidized to CO₂, but increased substrate concentrations can lead to an incomplete oxidation of organic substrates. For the utilization of fatty acids, this might be a result of a CoA transferase reaction as proposed for *Desulfo-*

bacterium autotrophicum (Schauder *et al.*, 1986), whereas acetate and propionate excretion after growth on lactate might be caused by partial fermentation of the lactate via a randomizing succinate pathway as described for *Desulfobulbus propionicus* (Stams *et al.*, 1984).

The 16S rRNA gene sequence analyses presented in this study demonstrate that strain MT-96^T is a member of the genus *Desulfacinum* with *Desulfacinum infernum*

as its closest relative (95.3% 16S rRNA sequence identity). Its physiological properties (Table 1), in combination with the results of the 16S rRNA gene sequence comparison, indicate that strain MT-96^T represents a novel species within the genus *Desulfacinum*. We propose the name *Desulfacinum hydrothermale* sp. nov. for this strain.

Ecological relevance

On the basis of its growth characteristics, i.e. its temperature optimum and its salt tolerance, strain MT-96^T seemed to be well adapted to the environment from which it was isolated. However, the pH range determined for the isolate does not cover the pH measured *in situ* at the sampling site. A possible explanation could be a toxic effect of H₂S at low pH within the batch cultures, which would not occur in the natural environment because of interaction with ferrous iron or by dilution due to exchange with the overlying water. However, strain MT-96^T may grow in microniches with favourable environmental conditions (Fortin *et al.*, 1996). The fractionation of sulfur isotopes during dissimilatory sulfate reduction obtained in batch cultures was within the range found for mesophilic sulfate reducers and reflected the *in situ* sulfur isotopic composition of the sediments (Böttcher *et al.*, 1999).

The finding of members of the genus *Desulfacinum*, represented by MT-96^T and M40/2 CIV-2.3, and of the genus *Thermodesulforhabdus*, represented by M40/2 CIV-3.2, at both vent sites indicates an important role for the degradation of organic compounds by thermophilic sulfate reduction at hydrothermal vents. The presence of thermophilic sulfate reducers has also been shown for a deep-sea vent site in Guaymas Basin (Jørgensen *et al.*, 1990, 1992). In addition, it seems plausible to assume that at least strain MT-96^T contributes to primary production at the shallow-water vent site by growing with H₂ and CO₂ because both substances are constituents of the hydrothermal fluid (Dando *et al.*, 1995). Our data further support the idea of Nilsen *et al.* (1996b), who proposed that species of the genera *Thermodesulforhabdus* and *Desulfacinum* are members of a deep hot biosphere (*sensu* Gold, 1992) that contains thermophilic and hyperthermophilic micro-organisms. However, until now these two genera had not been isolated from any environment other than oil reservoirs. The present findings extend their habitat range to marine hydrothermal vents. Moreover, at least *Thermodesulforhabdus* seems also to be present in Obsidian Pool, a freshwater hot spring in Yellowstone National Park (Hugenholtz *et al.*, 1998). The sequence OPB33 is closely affiliated with *Thermodesulforhabdus norvegicus* (Fig. 1) and was the second most abundant sequence in the clone library (Hugenholtz *et al.*, 1998). This suggests that these populations play an important role in this environment. Whilst this argues for the existence for a common subterranean biosphere, the

presence of *Thermodesulforhabdus norvegicus* and *Desulfacinum infernum* in oilfields could also be due to an intrusion of seawater that contained cells derived from hydrothermal sources and subsequent growth in the reservoir (Stetter *et al.*, 1993). There are only a few descriptions of thermophiles from deep-sea hydrothermal vents with maximal growth temperatures below 80 °C, although these organisms are thought to be abundant (Baross & Deming, 1995). Thus, it should be worthwhile to look for the presence of members of the *Desulfacinum*–*Thermodesulforhabdus* cluster at deep-sea vents, which are also thought to provide a window into the subsurface biosphere (Deming & Baross, 1993; Holden *et al.*, 1998; Summit & Baross, 1998).

Description of *Desulfacinum hydrothermale* sp. nov.

Desulfacinum hydrothermale (hy.dro.ther.ma'le. Gr. n. *hydor* water; Gr. n. *thermos* heat; M.L. neut. adj. *hydrothermale* pertaining to hot water, i.e. to hydrothermal vents).

Cells are oval or short rods, 0.8–1.0 by 1.5–2.5 µm. Spore formation is absent. Cells are motile, but motility can rapidly decline. Gram stain reaction of cells is negative. Strict anaerobe. Growth on H₂/CO₂, formate, acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, octanoate, decanoate, dodecanoate, hexadecanoate, octadecanoate, ethanol, propanol, butanol, hexanol, lactate and pyruvate. Substrate oxidation is usually complete, leading to production of CO₂, but at high substrate concentrations acetate can accumulate. Electron acceptors used are sulfate, sulfite and thiosulfate; nitrate and sulfur are not used. Slow fermentative growth on pyruvate. Addition of at least 15 g NaCl l⁻¹ is necessary. Optimum NaCl concentration for growth is between 32 and 36 g l⁻¹. NaCl is tolerated up to 78 g l⁻¹. Vitamins are not required for growth. Temperature requirements: *T*_{min}, 37 °C; *T*_{opt}, 60 °C; *T*_{max}, 64 °C. The pH range for growth is 6.0–7.5; the pH optimum is 7.0. The G + C content of the DNA is 59.5 mol %. The GenBank accession number for the 16S rRNA gene sequence is AF170417. The type strain is MT-96^T (= DSM 13146).

Emended description of the genus *Desulfacinum* (Rees *et al.* 1995, 88).

The sentence regarding motility should be changed to: 'Oval cells, which may be motile or non-motile'.

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