**Clostridium tepidiprofundi** sp. nov., a moderately thermophilic bacterium from a deep-sea hydrothermal vent

G. B. Slobodkina,1 T. V. Kolganova,2 T. P. Tourova,1 N. A. Kostrikina,1 C. Jeanthon,3 E. A. Bonch-Osmolovskaya1 and A. I. Slobodkin1

1Winogradsky Institute of Microbiology, Russian Academy of Sciences, Prospect 60-letiya Oktyabrya 7/2, 117312 Moscow, Russia
2Bioengineering Center, Russian Academy of Sciences, Prospect 60-letiya Oktyabrya 7/1, 117312 Moscow, Russia
3UMR 7144, Adaptation et Diversite en Milieu Marin (AD2M), CNRS and Universite Pierre and Marie Curie, Place Georges Teissier, 29682 Roscoff Cedex, France

A moderately thermophilic, anaerobic bacterium (strain SG 508T) was isolated from a hydrothermal vent chimney located at 13° N on the East Pacific Rise at a depth of 2650 m. Cells of strain SG 508T were straight to slightly curved rods, 0.4–0.6 μm in diameter and 2.0–3.0 μm in length. Spore formation was observed only below pH 5.5. The temperature range for growth was 22–60 °C, with optimum growth at 50 °C. The pH range for growth was 4.0–8.5, with optimum growth at pH 6.0–6.8. Growth of strain SG 508T was observed at NaCl concentrations ranging from 1.0 to 6.0 % (w/v), with optimum growth at 2.5 % (w/v). Substrates utilized by strain SG 508T included casein, peptone, tryptone, yeast extract, beef extract, starch, maltose and glucose. The products of glucose fermentation were ethanol, acetate, H2, formate and CO2. Strain SG 508T was able to reduce elemental sulfur to hydrogen sulfide. The DNA G+C content of strain SG 508T was 30.9 mol%. 16S rRNA gene sequence analysis revealed that the isolated organism belonged to cluster I of the genus Clostridium. On the basis of its physiological properties and data from phylogenetic analyses, strain SG 508T is considered to represent a novel species of the genus **Clostridium**, for which the name **Clostridium tepidiprofundi** sp. nov. is proposed. The type strain is SG 508T (=DSM 19306T =VKM B-2459T).

Among extreme environments, deep-sea hydrothermal vents contain large reservoirs of a wide variety of thermophilic and hyperthermophilic micro-organisms that belong to the Bacteria and Archaea. Micro-organisms from these environments use different metabolic pathways to grow; they may be chemolithoautotrophs, chemo-organoheterotrophs or mixotrophs (Jeanthon, 2000). Chemo-organotrophic, thermophilic, anaerobic bacteria isolated from these environments include members of the family Bacillaceae (L’Haridon et al., 2006) and the orders Thermotogales, ‘Thermooanaerobacteriales’ (Sokolova et al., 2001; Fardeau et al., 2004) and Clostridiales. The order Clostridiales includes a few thermophilic and moderately thermophilic species, namely *Caloranaerobacter azorenensis*, *Caminicella sporogenes*, *Tepidibacter thalassicus* and *Tepidibacter formicigenes* (Wery et al., 2004; Alain et al., 2002; Slobodkin et al., 2003; Urios et al., 2004); only one moderate thermophile isolated from these environments, *Clostridium caminithermale*, has been classified as a representative of the genus *Clostridium* (in cluster XI) (Brisbarre et al., 2003). Microbiological investigations of deep-sea hydrothermal vents have concentrated largely on thermophilic and hyperthermophilic micro-organisms and little attention has been paid to moderate thermophiles. In the present study, we report the isolation and characterization of an anaerobic, moderately thermophilic, fermentative bacterium (strain SG 508T) originating from a deep-sea hydrothermal vent. We suggest that this strain represents a novel species of the genus *Clostridium*.

Strain SG 508T was isolated from a sample of the outer part of a chimney-like structure (black smoker) covered with tubes and specimens of the polychaetous annelid *Alvinella* sp. The sample was collected during the AMISTAD cruise at the 13° N hydrothermal field on the East Pacific Rise at a depth of 2650 m as described by Slobodkin et al. (2001). An enrichment culture was initiated by inoculation of 10 % (w/v) of the sample into anaerobically prepared, bicarbonate-buffered, sterile (135 °C, 1 h) liquid medium of the following composition (per litre distilled water): 0.34 g...
KCl, 4 g MgCl₂, 6H₂O, 0.25 g NH₄Cl, 0.14 g CaCl₂, 2H₂O, 0.14 g K₂HPO₄, 18 g NaCl, 5 g NaHCO₃, 0.20 g yeast extract (Difco), 0.002 g Fe(NH₄)₂(SO₄)₂·7H₂O, 10 g casein (Hammerstein grade), 1 ml trace-element solution (Slobodkin et al., 1997), 10 ml vitamin solution (Wolin et al., 1963), 0.001 g resazurin and 0.50 g Na₂S·9H₂O (100 % gas-phase CO₂). A pure culture of strain SG 508ᵀ was obtained from a 50 °C enrichment culture by means of serial dilution in the same medium containing peptone (10 g l⁻¹) instead of casein followed by the selection of well-separated colonies that had developed in agar shake cultures (1.5 % agar in growth medium). Physiological studies on substrate and electron acceptor utilization and temperature, pH and salinity ranges for growth, light and electron microscopy, analytical techniques, DNA extraction and determination of G+C content were performed as described by Slobodkin et al. (1999). 16S rRNA gene amplification, sequencing and sequence analysis were performed as described by Zavarzina et al. (2002).

In agar-shake cultures, white, lens-shaped colonies (0.1–0.2 mm in diameter) of strain SG 508ᵀ appeared after 48–72 h incubation at 50 °C. Vegetative cells of strain SG 508ᵀ were straight to slightly curved rods, 0.4–0.6 μm in diameter and 2.0–3.0 μm in length. The cells occurred singly or in short chains, and had no flagella. When grown at below pH 5.5, strain SG 508ᵀ formed round, refractile endospores in terminally swollen sporangia. Ultrathin sectioning of cells of strain SG 508ᵀ revealed a distinct peptidoglycan layer in the cell wall.

The temperature range for growth of strain SG 508ᵀ was 22–60 °C, with optimum growth at 50 °C. No growth was detected at ≤20 or 62 °C after incubation for 3 weeks. The pH range for growth was 4.0–8.5, with optimum growth at between pH 6.5 and 6.8. No growth was detected at pH 3.5 or 8.9. Growth of strain SG 508ᵀ was observed at NaCl concentrations ranging from 1.0 to 6.0 % (w/v), with optimum growth at 2.5 % (w/v), but no growth was evident in the absence of NaCl or at 7.0 % (w/v). Substrates utilized by strain SG 508ᵀ included casein, peptone, tryptophan, yeast extract, beef extract, starch (each substrate at 10 g l⁻¹), maltose and glucose (25 mM each). Pyruvate, L-valine, L-arginine (25 mM each), DL-alanine (20 mM), L-proline (10 mM), DL-alanine (20 mM) + L-proline (10 mM), glycine (20 mM), DL-alanine (20 mM) + glycine (20 mM), fructose, xylose, cellobiose, sucrose, L-arabinose (25 mM each), glycerol, acetate, butyrate, lactate, formate, methanol, fumarate (20 mM each), betaine (5 mM), olive oil, xylan, CM-cellulose, filter paper, chitin (10 g l⁻¹ each) and H₂/CO₂ (80 : 20, v/v) were not utilized. All substrates were tested in the presence of 0.2 g yeast extract l⁻¹. In outgrown cultures in medium supplied with 25 mM glucose, the products of glucose fermentation were ethanol (3.6 mM), acetate (2.9 mM), H₂ (3.1 mM), formate (0.8 mM) and CO₂ (not quantitatively determined). Strain SG 508ᵀ reduced elemental sulfur (150 mM) to hydrogen sulfide with peptone (10 g l⁻¹) as an electron donor, but sulfur reduction did not stimulate growth. Strain SG 508ᵀ was not able to utilize nitrate, fumarate, sulfate, thiosulfate (20 mM each), sulfite (5 mM), amorphous iron(III) oxide (90 mM), iron(III) citrate (20 mM) or oxygen (20 %, v/v, in the gas phase) as electron acceptors with peptone (10 g l⁻¹) as an electron donor.

The G+C content of the genomic DNA of strain SG 508ᵀ was 30.9 mol% (Tm). A BLAST analysis revealed that strain SG 508ᵀ showed highest levels of 16S rRNA gene sequence similarity with members of the genus Clostridium within the low-G+C Gram-positive subdivision of the class Bacteria. A neighbour-joining comparison of 1464 nt of the 16S rRNA gene sequence of strain SG 508ᵀ with those available in the GenBank database showed that strain SG 508ᵀ belonged to cluster I of the genus Clostridium and related genera according to the nomenclature of Collins et al. (1994) (Fig. 1). Only 16S rRNA gene sequences of the type strains of recognized species were included in the analyses. The 16S rRNA gene sequence of strain SG 508ᵀ showed highest similarity to those of the type strains of Clostridium pascui (92 %) and Clostridium sporogenes (91 %) (Wilde et al., 1997; Olsen et al., 1995). Levels of 16S rRNA gene sequence similarity between strain SG 508ᵀ and the type strains of other members of phylogenetic cluster I were 90–91 %. Trees constructed based on the maximum-likelihood and maximum-parsimony algorithms displayed the same topology (data not shown). Transversion analysis (Woese et al., 1991) did not affect the phylogenetic position of the novel strain.

Cluster I of the genus Clostridium is the largest and the most phenotypically diverse of the clostridial groups. Although most members of this group are mesophiles, the group does include moderate thermophiles, namely Clostridium thermobutyricum and Clostridium thermopolalmarium (Wiegel et al.,...
1989; Soh et al., 1991), as well as a few psychrophilic species (Spring et al., 2003). Members of cluster I have been isolated from freshwater environments, with the exception of Clostridium oceanicum, which was isolated from marine sediments (Smith, 1970). Strain SG 508T shares morphological and physiological traits with members of cluster I of the genus Clostridium. The novel strain forms a separate branch on the phylogenetic tree and shows significant phylogenetic divergence from its closest relative, Clostridium pascui DSM 10365T (92% 16S rRNA gene sequence similarity). Strain SG 508T shows strictly anaerobic, organotrophic growth, and cells are rod-shaped and have the ability to form endospores. However, strain SG 508T is a moderately thermophilic marine bacterium, whereas its closest phylogenetic relatives are mesophilic bacteria that grow optimally in the absence of NaCl. On the basis of its phenotypic and physiological properties, and based on current taxonomic guidelines, we suggest that strain SG 508T represents a novel member of cluster I of the genus Clostridium, for which the name Clostridium tepidiprofundi sp. nov. is proposed.

Description of Clostridium tepidiprofundi sp. nov.

Clostridium tepidiprofundi (te.pi.di.pro.fun’.di. L. adj. tepidus moderately warm; L. n. profundus the depths of the ocean, N.L. gen. n. tepidiprofundi of the warm bottom of the ocean).

Cells are straight to slightly curved rods, 0.4–0.6 μm in diameter and 2.0–3.0 μm in length; spore formation is observed only at below PH 5.5. Cells form white lens-shaped colonies (0.1–0.2 mm in diameter) in agar-shake cultures. The temperature range for growth is 22–60 °C, with optimum growth at 50 °C. The pH range for growth is 4.0–8.5, with optimum growth at pH 6.0–6.8. Growth occurs at NaCl concentrations of 1.0–6.0% (w/v), with optimum growth at 2.5% (w/v). Anaerobic. Substrates utilized include casein, peptone, tryptone, yeast extract, beef extract, starch, maltose and glucose. Pyruvate, L-valine, L-arginine, DL-alanine, L-proline, DL-alanine plus L-proline, glycine, DL-alanine plus glycine, fructose, xylose, cellobiose, sucrose, L-arabinose, glycerol, acetate, butyrate, lactate, formate, methanol, fumarate, betaine, olive oil, xylan, CM-cellulose, filter paper, chitin and H2/CO2 are not utilized. The products of glucose fermentation are ethanol, acetate, H2, formate, and CO2. Reduces elemental sulfur to hydrogen sulfide. Is not able to utilize nitrate, fumarate, sulfate, sulfite, thiosulfate, amorphous iron(III) oxide, iron(III) citrate or oxygen (20%, w/v, in the gas phase) as electron acceptors. The DNA G+C content of the type strain is 30.9 mol% (Tm).

The type strain, SG 508T (= DSM 19306T =VKM B-2459T), was isolated from a deep-sea hydrothermal vent chimney located at 13° N on the East Pacific Rise.

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