Oceanotoga teriensis gen. nov., sp. nov., a thermophilic bacterium isolated from offshore oil-producing wells

Himali S. Jayasinghearachchi and Banwari Lal

Correspondence

Banwari Lal
banwaril@teri.res.in

The Energy and Resource Institute, Darbari Seth Block, Habitat Place, Lodhi Road,
New Delhi - 110 003, India

A novel, moderately thermophilic, chemo-organotrophic bacterium was isolated from formation fluid samples from an offshore oil-production well head at Bombay High (Western India). Cells were rod-shaped with a sheath-like outer structure ('toga'); the cells appeared singly, in pairs or in short chains. Cells grew at 25–70 °C (optimum 55–58 °C), pH 5.5–9.0 (optimum pH 7.3–7.8) and 0–12 % (w/v) NaCl (optimum 4.0–4.5 %). The isolate was able to grow on various carbohydrates or complex proteinaceous substances. The isolate reduced thiosulfate and elemental sulfur. The major end products of glucose fermentation were acetate, H₂ and CO₂. The DNA G + C content of the genomic DNA was 26.8 mol%. Phylogenetic analysis of the 16S rRNA gene placed the strain within the order *Thermotogales* in the bacterial domain. On the basis of 16S rRNA gene sequence comparisons and in combination with morphological and physiological characteristics, the isolate represents a novel species of new genus, for which the name *Oceanotoga teriensis* gen. nov., sp. nov. is proposed. The type strain of the type species is OCT74^T (=JCM 15580^T=LMG 24865^T).

Petroleum reservoirs constitute a group of unique sites with respect to their physico-chemical conditions (halophilic and thermophilic) and provide ecological niches from which taxonomically, physiologically and phylogenetically unusual microbes can be isolated (Bhupathiraju *et al.*, 1993; Ravot *et al.*, 1995; Fardeau *et al.*, 1997). Members of the order *Thermotogales*, first described as an order containing hyperthermophilic bacteria, have been isolated from extreme environments such as deep-sea hydrothermal vents, hot springs, continental and subcontinental volcanic areas, marine sediments and oil reservoirs (Nunoura *et al.*, 2007; Alain *et al.*, 2002; Huber & Stetter, 1992; Davey *et al.*, 1993).

Within the order *Thermotogales*, the genera *Thermotoga* (Huber *et al.*, 1986), *Thermosipho* (Huber *et al.*, 1989), *Fervidobacterium* (Patel *et al.*, 1985), *Petrotoga* and *Geotoga* (Davey *et al.*, 1993), *Marinitoga* (Wery *et al.*, 2001), *Kosmotoga* (DiPippo *et al.*, 2009) and *Thermococcoides* (Feng *et al.*, 2010) have been described. Representatives of these genera are anaerobic, chemo-organotrophic, thermophilic, Gram-negative, non-sporulating, rod-shaped bacteria that are characterized by a sheath-like outer structure called the 'toga' (Huber *et al.*, 1986). In this study, a novel strain of moderately thermophilic bacteria is described that

was isolated from offshore oil-producing well heads located at Bombay High (Western India).

Formation fluid samples were collected from oil-well heads into 50 ml anaerobic sterilized serum bottles containing sodium sulfide at a final concentration of 20 mM. Samples were transported to the laboratory under ambient conditions. Collection of samples was performed from December 2007 to January 2008.

A basal medium composed of (l⁻¹ deionized water): 1 g NH₄Cl₂, 0.3 g K₂HPO₄, 0.3 g KH₂PO₄, 0.2 g MgCl₂ · 6H₂O, 0.1 g CaCl₂ · 2H₂O, 10.0 g NaCl, 0.1 g KCl, 0.5 g NaC₂H₃O₂, 2.0 g yeast extract, 2.0 g bio-Trypticase, 1 mg resazurin and 10.0 ml trace mineral element solution (Balch *et al.*, 1979) was used for isolation and routine cultivation. The pH of the medium was adjusted to 7.5 with 1 M KOH at ambient temperature before sterilization (Ravot *et al.*, 1995).

The medium was boiled under a stream of O₂-free N₂ gas and aliquots of 6 ml and 10 ml were dispensed anaerobically into 10 ml and 60 ml serum bottles, respectively. The bottles were then sealed and autoclaved for 21 min at 121 °C. Prior to inoculation, 0.1 ml Na₂S · 9H₂O from a 2 % sterile anaerobic solution, 0.1 ml Na₂CO₃ from a 10 % anaerobic solution and 0.1 ml 20 mM filter-sterilized Na₂S₂O₃ were injected separately by syringe.

Formation fluid samples (0.1 ml) were inoculated into the anaerobic basal medium described above. All serum bottles were incubated at 40 °C for 3–5 days depending on

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of *Oceanotoga teriensis* OCT74^T is EU588727.

growth. Subculturing was conducted in the same medium after 3 days of incubation. All the subcultures were incubated at 40 °C under atmospheric pressure. Three days after incubation, the first sets of subcultures were serially diluted using fresh anaerobic basal medium and 10 µl aliquots of the serially diluted culture (10^{-7} and 10^{-8} dilutions) were streaked on the same basal medium that had been solidified with 1.2 % agar. All the culture plates were incubated at 40 °C in an anaerobic chamber ($\text{N}_2:\text{H}_2:\text{CO}_2$; 90:5:5) for 2–4 days until colonies appeared. Well-isolated colonies were transferred anaerobically to the liquid basal medium and incubated as described above. The plating and single colony isolation procedure was repeated several times until a pure culture of strain OCT74^T was obtained. Gram-staining was routinely performed and the purity of the cultures was examined under a light microscope (AX 10, Carl Zeiss). Light microscopic examinations revealed the presence of rod-shaped bacteria with an outer sheath-like structure (toga) and a Gram-negative appearance. The morphology of strain OCT74^T was examined further by transmission electron microscopy (Morgagni-268D, Fei). Cells were negatively stained as described previously (Zillig *et al.*, 1990).

Cells of strain OCT74^T were rod-shaped with a diameter of 0.5–0.7 µm and length of 1.5–1.7 µm (Fig. 1a). Cells occurred singly, in pairs and in short irregularly curved chains surrounded by the outer sheath-like structure, or toga, that is specific to members of the order *Thermotogales*. The number of cells in the chains varied depending on the culture conditions. Cells were Gram-negative in appearance in all the growth phases. The cell envelope was clearly visible by TEM in cells at all phases of growth. In addition, TEM images revealed the presence of multiple flagella (Fig. 1a, b). In the stationary growth phase, the cells became short rods or spheres surrounded by a sheath (Fig. 1c). Cells were capable of multiplying within the sheath and formed enlarged structures. Spores were not observed.

Substrate utilization tests were performed in anaerobically prepared MMS medium (Huber *et al.*, 1986) containing (1^{-1}): 6.93 g NaCl, 1.75 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 1.38 g $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.16 g KCl, 25 mg NaBr, 7.5 mg H_3BO_3 , 3.8 mg $\text{SrCl}_2 \cdot 6\text{H}_2\text{O}$, 0.38 g CaCl_2 , 0.5 g KH_2PO_4 , 0.5 g Na_2S , 2 mg $(\text{NH}_4)_2\text{Ni}(\text{SO}_4)_2$, 15 ml trace minerals (Balch *et al.*, 1979) and 1 mg resazurin. The following substrates were tested at a final concentration of 20 mM: glucose, fructose, ribose, rhamnose, raffinose, arabinose, maltose, cellobiose, cellulose, pectin, xylan, xylose, starch, sorbitol, formate, acetate and ethanol. Growth of the strain on acetate, ethanol and formate was tested in the presence of thiosulfate and elemental sulfur. The following proteinaceous substances were also tested at 0.2 % (w/v): yeast extract, brain-heart infusion, peptone, tryptone and casein. The pH of the medium was adjusted to 7.5 with 1 M NaOH. All substrate utilization studies were performed in triplicate using 60 ml serum bottles containing 10 ml MMS medium. Bacterial growth and substrate utilization were determined by viable colony counts on solid medium and by monitoring the turbidity at OD₆₀₀ by spectrometry (UV-2450, Shimadzu). Positive cultures were confirmed by subculturing on the same basal medium.

Strain OCT74^T possessed a chemo-organotrophic metabolism and was able to grow on the following substances: glucose, fructose, cellobiose, arabinose, raffinose, rhamnose, sucrose, xylose, ribose, starch, ethanol, formate, acetate, brain heart infusion, yeast extract and bio-Trypticase. Growth was not observed in cultures with lactose, maltose, pectin, sorbitol or casein added. Growth on carbohydrates was found to be stimulated by yeast extract.

The temperature range for growth and the tolerance of different NaCl concentrations were determined in liquid MMS medium supplemented with 20 mM glucose. The pH of the medium was adjusted to pH 7.5. The requirement for NaCl for growth was tested in liquid MMS medium containing 0, 0.05, 0.5, 1, 2, 3, 4, 5, 7, 10 and 12 % NaCl (w/v). Tolerance to NaCl was studied using a modified

Fig. 1. (a–b), TEM images of negatively stained rod-shaped cells surrounded by a sheath-like structure or ‘toga’ in the mid-exponential phase of growth. (c), TEM image of a negatively stained spherically shaped cell in the stationary phase of growth. Bars, 2 µm.

MMS medium in which NaHCO_3 was replaced with K_2CO_3 and Na_2S was replaced with K_2S . The pH range for growth was studied in liquid MMS medium. The pH of the medium was adjusted to different pH values ranging from 4.5 to 9.5 with 1 M HCl or 1 M KOH.

Strain OCT74^T grew anaerobically and growth occurred from 25 to 70 °C. However, weak growth was observed at 70 °C. The strain was readily adaptable to mesophilic growth (37 °C) after several subculturing steps under mesophilic conditions. Growth of strain OCT74^T was observed within the pH range 5.5–9.0 with an optimum pH for growth of 7.3–7.8. Strain OCT74^T grew within the NaCl concentration range of 0–12 % with an optimum of 4.0–4.5 %. Under optimal growth conditions, growth of the novel strain at high concentrations of NaCl (>7 %) varied according to the carbon source used. Weaker growth was observed at 10–12 % (w/v) NaCl even in the presence of preferred carbon sources such as fructose or dextrin. Under optimal conditions, the doubling time for strain OCT74^T was about 1.6 h⁻¹ in MMS medium with glucose as the sole carbon source.

The utilization of various electron acceptors was studied in liquid MMS medium containing 3 g glucose l⁻¹ as the electron donor. Cultures grown in pre-reduced MMS medium without added electron acceptor were used as inocula (5 % v/v). Electron acceptors were added to the medium in the form of filter-sterilized (0.2 µm, Millipore) or autoclaved stock solutions. The final concentrations of the electron acceptors (FeSO_4 , NH_4SO_4 , KNO_3 and $\text{Na}_2\text{S}_2\text{O}_3$) were 20 mM. Elemental sulfur (50 mg) was added to individual serum bottles containing 10 ml liquid basal medium prior to autoclaving.

Hydrogen sulfide production was detected colorimetrically using a spectrophotometer (U2000, Hitachi) at 600 nm using a sulfide detection kit (Merck) according to the manufacturer's instructions. Strain OCT74^T could utilize sodium thiosulfate and elementary sulfur as electron acceptors, which resulted in the production of hydrogen sulfide. The addition of elemental sulfur could support the growth of the strain OCT74^T. Thiosulfate and sulfur were both reported to improve the growth rates or cell yields for *Fervidobacterium islandicum*, *Thermotoga maritima*, *Thermotoga neapolitana*, *Thermosipho africanus* and *Thermotoga elfii* (Ravot *et al.*, 1995). However, in the case of *Marinitoga camini*, neither sulfur nor thiosulfate had a major stimulatory effect on growth rates and final cell concentrations (Wery *et al.*, 2001). For strain OCT74^T, L-cystine hydrochloride did not support growth and growth was suppressed by higher concentrations of this substance.

The end products of glucose metabolism in the liquid phase were determined by GC using a machine (GC6890N, Agilent) equipped with a flame-ionization detector and a DB-WAXetr column (30 m × 530 µm × 1 µm). The oven temperature was 140 °C and the temperature ramping was 1 °C min⁻¹ up to 158 °C. The injector and detector temperatures were 220 °C and 230 °C, respectively. Helium

was used as the carrier gas. Ethanol was detected by HPLC using a machine (1100 series, Agilent) equipped with a Sugar-Pak I column (Water Research). The composition of the headspace gases from the cultures was directly measured by GC with a GC6890N (Agilent) machine equipped with a thermal conductivity detector and a HP-PLOT Q column (15 m × 530 µm × 40 µm). The oven, injector and detector temperatures were 40, 60 and 250 °C, respectively, and nitrogen was used as the carrier gas. The major end products of glucose fermentation were acetate, CO_2 and H_2 with minor production of ethanol.

Identification and quantification of fatty acid methyl esters was performed using the Sherlock Microbial Identification System (MIS) from MIDI Inc. (USA). The assay was conducted twice. The major fatty acids detected in cell extracts of strain OCT74^T were $\text{C}_{16:0}$, $\text{C}_{17:0}$ cyclopropane, $\text{C}_{16:1}\omega 7c$, $\text{C}_{18:1}\omega 7c$, $\text{C}_{14:0}$, $\text{C}_{16:0}$ 3-OH (Table 1).

Antibiotic susceptibility tests were performed with anaerobic filter-sterilized stocks of rifamycin, vancomycin,

Table 1. Fatty acid contents (%) of strain OCT74^T

tr, Trace (<0.1 %). The data presented in the table are the means from two independent experiments.

Fatty acid	Total (%)
$\text{C}_{10:0}$ 3-OH	0.2
$\text{C}_{12:0}$	0.3
$\text{C}_{14:0}$	5.6
$\text{C}_{14:0}$ DMA	tr
$\text{C}_{15:1}\omega 5c$	0.2
$\text{C}_{15:0}$	0.5
$\text{C}_{16:1}\omega 7c$	8.5
$\text{C}_{16:1}\omega 5c$	0.6
$\text{C}_{16:0}$	27.5
$\text{C}_{15:0}$ 3-OH	0.3
$\text{C}_{17:0}$ cyclopropane	14.0
$\text{C}_{17:0}$	1.1
$\text{C}_{16:0}$ 2-OH	2.0
$\text{C}_{16:0}$ 3-OH	5.7
$\text{C}_{18:1}\omega 5c$	0.4
$\text{C}_{18:0}$	1.0
Unknown*	17.8
Summed features†	
1	0.6
2	0.3
5	5.9
10	15.8
13	0.3

*Unknown fatty acids; these compounds have no names listed in the Peak Library File of the MIDI system and are therefore not identified.

†Summed features represent fatty acids that cannot be separated by the Microbial Identification System. Summed feature 1 consisted of $\text{C}_{14:0}$ aldehyde; summed feature 2 consisted of $\text{C}_{12:0}$ 3-OH; summed feature 5 consisted of $\text{C}_{14:0}$ 3-OH; summed feature 10 consisted of $\text{C}_{18:1}\omega 7c$; summed feature 13 consisted of $\text{C}_{14:0}$ 2-OH.

chloramphenicol, kanamycin (each at final concentrations of 10 and 100 $\mu\text{g ml}^{-1}$). Growth was inhibited by spectinomycin, rifamycin and chloramphenicol at 25 $\mu\text{g ml}^{-1}$. The strain was sensitive to vancomycin at 100 $\mu\text{g ml}^{-1}$. Growth of strain OCT74^T was inhibited at kanamycin concentrations of 250 $\mu\text{g ml}^{-1}$.

Genomic DNA was isolated by a standard phenol/chloroform extraction followed by ethanol precipitation (Sambrook *et al.*, 1989). The DNA base composition was determined using the HPLC technique (Mesbah *et al.*, 1989). The G + C content of the genomic DNA of the strain OCT74^T was determined as 26.8 mol%.

The 16S rRNA gene of strain OCT74^T was amplified by PCR using MicroSec Full Gene kit (Applied Biosystems) according to the manufacturer's instructions. The amplified fragment was extracted from a 1.5 % agarose gel using the Qiagen Gel extraction kit (Qiagen). Purified PCR products were sequenced using the DyeDeoxy Terminator Cycle sequencing kit (Applied Biosystems) as directed in the manufacturer's protocol with an automatic DNA sequencer (model 310 ABI PRISM, Applied Biosystems).

To ascertain the phylogenetic position of the novel strain, the 16S rRNA gene sequence of strain OCT74^T was compared with the most similar sequences available in GenBank using the BLAST programme (Altschul *et al.*, 1997). A BLAST search against the GenBank database

revealed that strain OCT74^T belonged to the order *Thermotogales*. Consequently, the 16S rRNA gene sequence of strain OCT74^T was aligned with closely related sequences and those of representatives of the other genera of the order *Thermotogales* that were retrieved from GenBank using CLUSTAL W (Thompson *et al.*, 1994). Pairwise evolutionary distances were computed using the Jukes–Cantor model (Jukes & Cantor, 1969). Phylogenetic analyses were performed using PHYLIP version 3.67 (Felsenstein, 2007) and an unrooted phylogenetic tree was constructed using the neighbour-joining algorithm (Saitou & Nei, 1987). Confidence in the tree topologies was evaluated by bootstrap analysis (Felsenstein, 1985) based on 100 resamplings.

Of the genera within the order *Thermotogales*, strain OCT74^T was closest to the genera *Geotoga*, *Petrotoga* and *Marinitoga*. The sequence of strain OCT74^T grouped with the *Geotoga*, *Petrotoga* and *Marinitoga* lineage with all three algorithms used (neighbour-joining, maximum-parsimony and maximum-likelihood). However, within this lineage, strain OCT74^T represented a distinct branching line (Fig. 2).

The level of 16S rRNA gene sequence similarity between strain OCT74^T and recognized members of the related genera was less than 88 % in all the cases. The highest 16S rRNA gene sequence similarity (88 %) was to *Geotoga petraea* and *Geotoga subterranea*. Similarity

Fig. 2. 16S rRNA gene sequence-based phylogenetic distance-analysis dendrogram indicating the position of strain OCT74^T among closely related members of the order *Thermotogales*. Numbers at branching points refer to bootstrap values. The position of the root was determined by including *Aquifex pyrophilus* as the outgroup. Bar, 0.05 substitutions per nucleotide position.

values between 80 and 84 % were found between strain OCT74^T and members of the genera *Petrotoga* and *Marinitoga*.

Strain OCT74^T was isolated from formation fluid samples collected from offshore oil-producing well heads. The oil wells were repeatedly flooded with seawater for secondary oil recovery and so the formation fluid was partly mixed with seawater. Given that strain OCT74^T was tolerant to a wide range of salinities, it could have originated from seawater. The novel strain was moderately thermophilic, but could be readily adapted to mesophilic growth conditions after several subculturing steps under mesophilic conditions (37 °C). The closest phylogenetic neighbours to strain OCT74^T, based on 16S rRNA gene sequence analysis, were *G. petraea* and *G. subterranea*, but these strains showed around 12 % sequence divergence from the novel strain. In addition to distinct phylogenetic and genomic differences between strain OCT74^T and species of the genus *Geotoga*, several differential phenotypic characteristics were also found. Unlike members of the genus *Geotoga*, strain OCT74^T was able to grow with 0 % NaCl (Davey *et al.*, 1993). Further, in contrast to species of the genus *Geotoga*, strain OCT74^T was able to utilize xylose, raffinose, acetate, formate and ethanol (weak growth) as sole carbon sources. Yeast extract supported the growth of strain OCT74^T. Cells of the novel strain were able to grow and replicate within the sheath and formed rotund bodies as described for *Fervidobacterium nodosum*. This characteristic has not been reported for members of the genera *Geotoga*, *Petrotoga* or *Marinitoga* (Davey *et al.*, 1993).

The 16S rRNA gene sequence divergence of strain OCT74^T with members of the genera *Petrotoga* and *Marinitoga* was >15 %. Therefore, phenotypic comparisons with recognized species of these genera were not performed. As shown in Table 2, numerous phenotypic differences were found between strain OCT74^T and other members of the genera of the order *Thermotogales*. In addition, strain OCT74^T was distinctly different from members of genus *Fervidobacterium*, which produce spheroids due to the protrusion of the outer cell wall (Patel *et al.*, 1985). Strain OCT74^T had a low optimum temperature for growth and so could be distinguished from members of the genera *Thermotoga*, *Fervidobacterium* and *Thermosipho*, which have much higher optimum temperatures (above 60 °C) for growth (Huber *et al.*, 1986, 1989; Patel *et al.*, 1985). Moreover, the DNA G + C content of strain OCT74^T was 26.8 mol%, which was lower than those of *G. petraea* and *G. subterranea* (30 mol%), *Petrotoga miotherma* (40 mol%), members of the genus *Thermotoga* (40–46 mol%), *F. islandicum* (40 mol%) and *Thermosipho africanus* (30 mol%). Unlike species of the genus *Petrotoga*, which are described as fermentative bacteria able to grow on various polysaccharides, strain OCT74^T was able to grow on both sugars and complex proteinaceous substances such as yeast extract, peptone, tryptone and brain-heart infusion.

Based on comparative morphological, physiological and genomic data, it is concluded that strain OCT74^T represents a novel species of a new genus, for which the name *Oceanotoga teriensis* gen. nov., sp. nov. is proposed.

Description of *Oceanotoga* gen. nov.

Oceanotoga (O.ce.a.no.to'ga. Gr. n. *okeanos* the ocean; L. fem. n. *toga* a covering, garment; N.L. fem. n. *Oceanotoga* an ocean toga, referring to the isolation of the organism from the ocean and the presence of a 'toga' sheath-like outer cover).

Cells are rod-shaped with a sheath-like outer structure. Cells occur singly, in pairs or in chains. Cells are Gram-negative, no spores are formed. During the stationary phase of growth, the rods form spherical structures that are still surrounded by the sheath. Growth is anaerobic, moderately thermophilic and moderately halophilic. Chemo-organotrophic and able to ferment a broad spectrum of carbohydrates and various proteinaceous substances. Able to reduce thiosulfate and elemental sulphur to H₂S, but these compounds are not essential for growth. The DNA G + C content of the type strain of the type species is 26.8 mol%. The 16S rRNA gene sequence comparisons place the genus *Oceanotoga* in the bacterial domain within the order *Thermotogales*, close to the *Geotoga* lineage. The type species is *Oceanotoga teriensis*.

Description of *Oceanotoga teriensis* sp. nov.

Oceanotoga teriensis [te.ri.en'sis. N.L. fem. adj. *teriensis* pertaining to TERI, named in honour of The Energy and Resource Institute (TERI), New Delhi, India].

Has the following characteristics in addition to those given in the genus description. Under optimum conditions, cells appear as short rods (2–3 µm long × 0.5–1 µm wide) and form pairs or chains. The length of the chains is variable. Cells are motile with multiple flagella. No spores are formed. Growth occurs between 25 and 70 °C (optimum, 55–58 °C), at pH values between pH 5.5 and 9.0 (optimum, pH 7.3–7.8) and NaCl concentrations between 0 and 12 % (w/v) (optimum 4.0–4.5 % NaCl). The doubling time under optimal conditions is approximately 1.6 h. Chemo-organotrophic and able to grow on glucose, fructose, starch, cellobiose, arabinose, raffinose, xylan, rhamnose, sucrose, xylose, ribose and complex proteinaceous substances such as brain-heart infusion, bio-Trypticase and peptone in the presence of yeast extract. Reduces thiosulfate and elemental sulfur to sulfide. The end products of glucose fermentation are H₂, CO₂ and acetic acid. The 16S rRNA gene sequence similarity to *G. petraea* and *G. subterranea* is approx. 88 %.

The type strain, OCT74^T (=JCM 15580^T=LMG 24865^T), was isolated from oil-producing well heads located at Bombay High, Western India. The DNA G + C content of the type strain is 26.8 mol%.

Table 2. Differential characteristics between strain OCT74^T and related genera of the order *Thermotogales*

Taxa: 1, OCT74^T (data from this study); 2, *G. subterranea* ATCC 51225^T; 3, *G. petraea* ATCC 51226^T (data from Davey *et al.*, 1993); 4, *P. miotherma* ATCC 51224^T (Davey *et al.*, 1993); 5, *M. camini* DSM 13578^T (Wery *et al.*, 2001); 6, *F. nodosum* ATCC 35602^T (Patel *et al.*, 1985); 7, *Thermotoga maritima* DSM 3109^T (Huber *et al.*, 1986). NR, not reported; +, utilized; –, not utilized; +/-, variable.

Characteristic	1	2	3	4	5	6	7
Source	Oil-producing well, Bombay High, India	Oilfields (TX & OK, USA)	Oilfields (TX & OK, USA)	Oilfields (TX & OK, USA)	Deep-sea hydrothermal chimney, Mid-Atlantic Ridge	Hot spring (New Zealand)	Various geothermally heated locations on the sea floor, Italy & the Azores
Temp. range (°C)	25–70	30–60	30–55	35–65	25–65	40–80	55–90
Optimum temp. (°C)	55–58	45	50	55	55	65–70	80
pH range	5.5–9.0	5.5–9.0	5.5–9.0	5.5–9.0	5–9	6–8	5.5–9
Optimum pH	7.3–7.8	6.5	6.5	6.5	7	7–7.5	NR
NaCl range (% w/v)	0–12	0.5–10	0.5–10	0.5–10	1.0–4.5	NR	0.25–3.75
Optimum NaCl (% w/v)	4.0–4.5	4	3	2	2	NR	NR
DNA G + C content (mol%)	26.8	30	30	40	29	33.7	46
Reduction of S ⁰	+	+	+	+	+	NR	+
Reduction of S ₂ O ₃ ^{2–}	+	NR	NR	NR	–	NR	NR
Flagella	+	+	+	–	+	+	+
Utilization of							
Mannose	–/+	+	+	+	NR	+	NR
Lactose	–	+	+	+	NR	+	NR
Maltose	–	+	+	+	+	+	+
Xylose	+	–	–	NR	NR	–	+
Raffinose	+	–	–	–	NR	+	NR
Pectin	–	NR	NR	NR	+	+	–
Starch	+	+	+	+	NR	NR	+
Ribose	+	NR	NR	NR	NR	–	+
Cellulose	+	NR	NR	NR	+	–	–
Sorbitol	–	NR	NR	NR	NR	+	NR
Ethanol	+	– (slow)	–	–	NR	NR	NR
Diagnostic fermentation product(s) from glucose	H ₂ , CO ₂ , acetate, ethanol	H ₂ , CO ₂ , acetate and ethanol	H ₂ , CO ₂ , acetate and ethanol	H ₂ , CO ₂ , acetate and ethanol	Acetate, lactate, isovalerate, isobutyrate, H ₂ , CO ₂ , H ₂ S	NR	Lactate, acetate, CO ₂ , H ₂

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