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CHAPTER 12.1

Thermotogales

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Introduction

Members of the Thermotogales represent a very deep phylogenetic branch within the 16S rRNA gene tree. Within the order, members of the genus *Thermotoga* have an upper temperature border of growth at 90°C and represent, together with members of the order *Aquificales*, the bacteria with the highest growth temperatures known so far. Representatives of the Thermotogales are widespread and cosmopolitan, and they thrive mainly in volcanically or geothermally heated environments. Owing to their strictly organotrophic way of life, they are consumers of microbial biomaterial within high temperature ecosystems.

For the Thermotogales, the following order criteria are characteristic: thermophilic, non-sporeforming, rod-shaped cells with an outer sheath-like envelope ('toga'); Gram-negative, but *meso*-diaminopimelic acid not present in the peptidoglycan; strictly anaerobic, fermentative bacteria; acetate, carbon dioxide, and hydrogen metabolites from glucose fermentation; inhibition of growth by hydrogen; lysozyme-sensitive; unusual long-chain dicarboxylic fatty acids present in the lipids; and G+C content, 29–50 mol%.

Phylogeny

By 16S rRNA gene sequence comparison it was shown first in 1987 that *Thermotoga maritima* represents a slowly evolving lineage and a deep phylogenetic branch within the bacterial domain (Huber et al., 1986; Woese, 1987; Fig. 1). On the basis of this result, a thermophilic origin of the bacterial domain was proposed (Achenbach-Richter et al., 1987). The outstanding phylogenetic placement of *T. maritima* was strengthened by comparative analysis of other macromolecules such as 23S rRNA, elongation factor Tu and G, ß-subunit of the ATPase, *fus* gene and of ferredoxins (Bachleitner et al., 1989; Schleifer and Ludwig, 1989; Tiboni et al., 1991; Ludwig et al., 1993; Blamey et al., 1994; Darimont and Sterner, 1994) and by whole genome-based phylogenetic analysis (Fitz-Gibbon and House, 1999). In contrast, sequence comparisons of bacterial RNA-polymerase large subunits placed *T. maritima* next to the chloroplasts, and the analysis of the bacterial DNA polymerase III, class II, placed *T. maritima* next to *Clostridium acetobutylicum* (Palm et al., 1993). Furthermore, a 16S rRNA phylogenetic tree based on the most conserved positions proposed that the Planctomycetales is the first branching bacterial group and not a hyperthermophilic bacterium (Brochier and Philippe, 2002). On the basis of conserved inserts and deletions found in various proteins, Gupta and Griffith (2002) placed *T. maritima* phylogenetically within the Gram-positive Bacteria. However, owing to biochemical and ultrastructural features, *T. maritima* is considered to be a true Gram-negative bacterium (see also Identification).

Taxonomy

Owing to the outstanding phylogenetic position of *T. maritima* in combination with unique morphological, physiological and biochemical criteria, the order Thermotogales consisting of the single family Thermotogaceae was described (Huber et al., 1992b). Within the Thermotogaceae, the genera *Thermotoga* (Huber et al., 1986; Huber and Stetter, 2001b), *Thermosipho* (Huber et al., 1989a; Ravot et al., 1996b; Huber and Stetter, 2001b), *Fervidobacterium* (Patel et al., 1985a; Huber et al., 1990; Huber and Stetter, 2001b), *Geotoga*, *Petrotoga* (Davey et al., 1993a; Davey et al., 2001a; Davey et al., 2001b) and *Marinitoga* (Wery et al., 2001) have been described. The species belonging to the different genera of the Thermotogales are listed in Table 1; their phylogenetic relationship is shown in Fig. 2. On the basis of 16S rRNA gene sequence analysis, *T. maritima*, *Thermotoga neapolitana*,

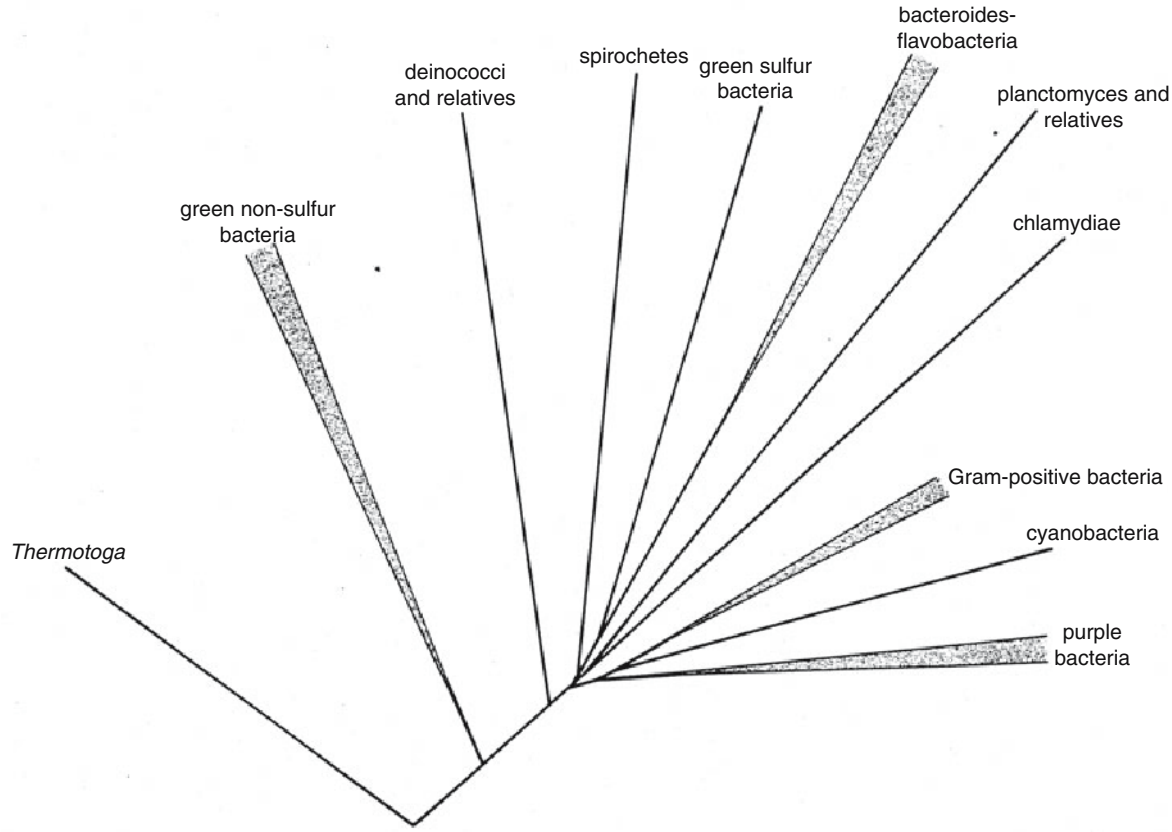

Fig. 1. Phylogenetic tree based upon 16S rRNA gene sequence comparison showing the phylogenetic position of the species *Thermotoga maritima* at the root of bacterial tree. (From Woese, 1987; courtesy of C. W. Woese.)

Thermotoga petrophila and *Thermotoga naphthophila* show a very close relationship (Fig. 2). However, based on specific sequence signatures in the 16S rRNA gene (*Escherichia coli* numbering; Brosius et al., 1981; Woese, 1987), the *T. maritima – T. neapolitana* group (position 1031, C; 1290, G; 1364, U) can be distinguished from the *T. naphthophila – T. petrophila* group (position 1031, U; 1290, A; 1364, C). Recently, the phylum Thermotogae, consisting of the single class Thermotogae was proposed (Reysenbach, 2001).

Habitat

Members of the Thermotogales are widespread and cosmopolitan. They thrive within continental solfatara springs of low salinity, shallow and deep-sea marine hydrothermal systems and high-temperature marine and continental oil fields.

From the genus *Thermotoga*, the type species *T. maritima* had been originally isolated from a geothermally heated, shallow marine sediment at Vulcano, Italy (Huber et al., 1986). The second species of this genus, *T. neapolitana*, was obtained from a submarine thermal vent at Lucrino near Naples, Italy (Belkin et al., 1986; Jannasch et al., 1988). Members of the marine *T. maritima – T. neapolitana* group are widespread within high temperature ecosystems and have also been isolated from shallow submarine hydrothermal systems on Sangeang Island (Indonesia), Ribeira Quente, Sao Miguel Island (the Azores), Kunashir Island (north of Japan) and the Fiji Island (Huber and Stetter, 1992b). Additional isolates were obtained from the Kolbeinsey Ridge north of Iceland in a depth of 106 m and from deep-sea hot sediments in a depth of 2000 m (Guaymas, Mexico; Huber and Stetter, 1992a; Huber and Stetter, 1992b). *Thermotoga thermarum* was originally isolated from continental solfataras with low ionic strength at Lac Abbé, Djibouti, Africa (Windberger et al., 1989). The closest cultivated relative of *T. thermarum* is the so far undescribed new *Thermotoga* species BB13-1-L6A, which thrives in the geothermally heated water (68°C; pH 6.8)

Table 1. List of the described species within the Thermotogales.

Species	Culture collection	Accession number	Effective publication	Validation
Thermotoga maritima MSB8	DSM 3109 and ATCC 43589	M21774	Huber et al., 1986	Stetter and Huber, 1986
Thermotoga neapolitana NS-E	DSM 4359 and ATCC 49049	AB039768	Jannasch et al., 1988	Jannasch et al., 1989
Thermotoga thermarum LA3	DSM 5069	AB039769	Windberger et al., 1989	Windberger et al., 1992
Thermotoga elfii SEBR 6459	DSM 9442 and ATCC 51869	X80790	Ravot et al., 1995	Ravot et al., 1995
Thermotoga subterranea SL1	DSM 9912	U22664	Jeanthon et al., 1995	Jeanthon et al., 2000
Thermotoga hypogea SEBR 7054	DSM 11164	U89768	Fardeau et al., 1997	Fardeau et al., 1997
Thermotoga petrophila RKU-1	DSM 13995, ATCC BAA-488, and JCM 10881	AB027016	Takahata et al., 2001	Takahata et al., 2001
Thermotoga naphthophila RKU-10	DSM 13996, ATCC BAA-489, and JCM 10882	AB027017	Takahata et al., 2001	Takahata et al., 2001
Thermotoga lettingae TMO	DSM 14385 and ATCC BAA-301	AF355615	Balk et al., 2002	Balk et al., 2002
Thermosipho africanus Ob7	DSM5309	M24022	Huber et al., 1989a	Huber et al., 1989b
Thermosipho melanesiensis BI429	DSM 12029 and CIP 104789	Z70248	Antoine et al., 1997	Antoine et al., 1997
Thermosipho japonicus IHB1	DSM 13481 and JCM 10495	AB024932	Takai und Horikoshi, 2000a	Takai and Horikoshi, 2000b
Thermosipho geolei SL31	DSM 13256 and JCM 10986	AJ272022	L'Haridon et al., 2001	L'Haridon et al., 2001
Fervidobacterium nodosum Rt17-B	DSM 5306 and ATCC 35602	M59177	Patel et al., 1985a	Patel et al., 1985b
Fervidobacterium islandicum H21	DSM 5733 and ATCC 49647	M59176	Huber et al., 1990	Huber et al., 1991
Fervidobacterium gondwanense AB39	DSM 13020 and ACM 5017	Z49117	Andrews and Patel, 1996	Andrews and Patel, 1996
Fervidobacterium pennivorans Ven5	DSM 9078		Friedrich and Antranikian, 1996	Friedrich and Antranikian, 1999
Geotoga petraea T5	ATCC 51226	L10658	Davey et al., 1993a	Davey et al., 1993b
Geotoga subterranea CC-1	ATCC 51225	L10659	Davey et al., 1993a	Davey et al., 1993b
Petrotoga miotherma 42-6	DSM 10691 and ATCC 51224	L10657	Davey et al., 1993a	Davey et al., 1993b
Petrotoga mobilis SJ95	DSM 10674	Y15479	Lien et al., 1998	Lien et al., 1998
Petrotoga olearia SL24	DSM 13574 and JCM 11234	AJ311703	L'Haridon et al., 2002	L'Haridon et al., 2002
Petrotoga sibirica SL25	DSM 13575 and JCM 11235	AJ311702	L'Haridon et al., 2002	L'Haridon et al., 2002
Marinitoga camini MV 1075	DSM 13578 and CNCM I-2413	AJ250439	Wery et al., 2001	Wery et al., 2001
Marinitoga piezophila KA3	DSM 14283 and JCM 11233	AF326121	Alain et al., 2002	Alain et al., 2002

Abbreviations: DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig, Germany; JCM, Japan Collection of Microorganisms, RIKEN, Saitama, Japan; ATCC, American Type Culture Collection, Manassas, VA, United States; CNCM, Collection Nationale de Cultures de Microorganismes, Paris, France; ACM, University of Queensland Microbial Culture Collection, Australia; and CIP, Collection de L'Institut Pasteur, Paris, France.

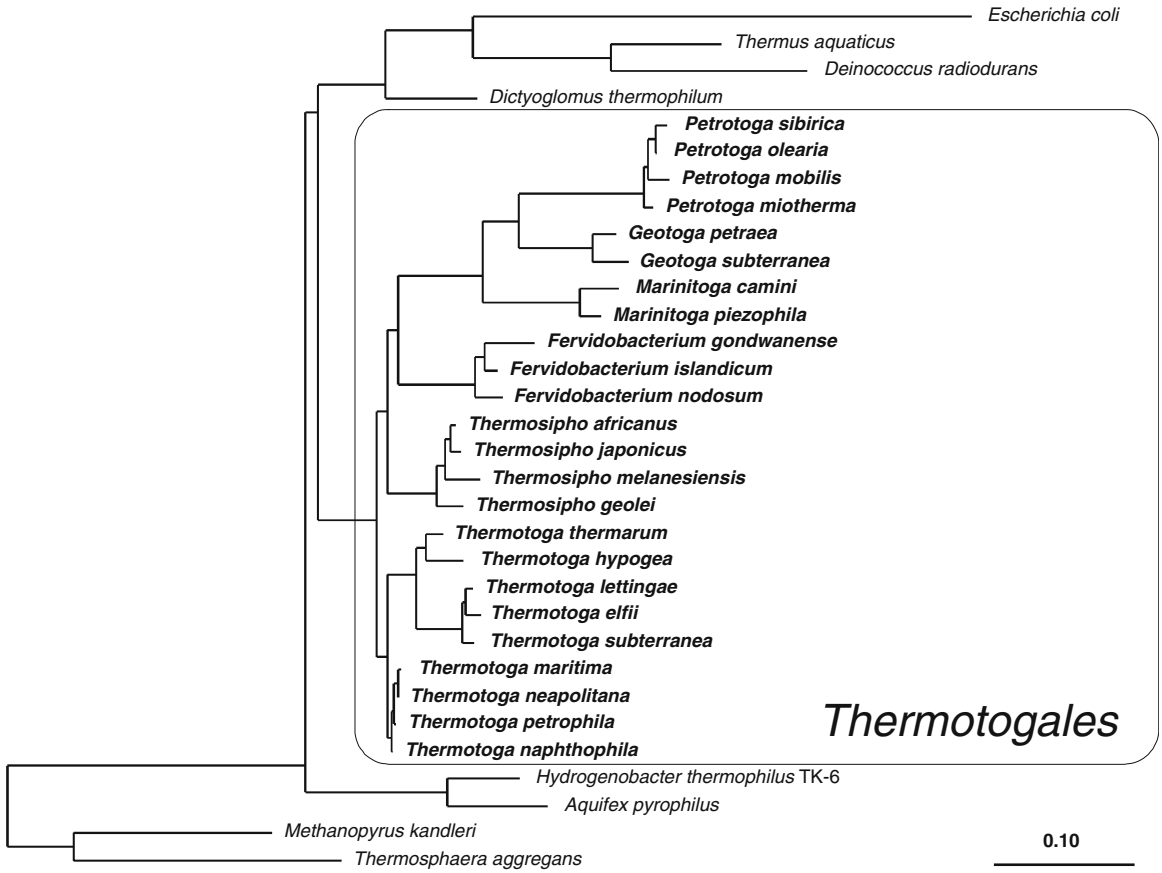

Fig. 2. 16S rRNA-based phylogenetic tree of the Thermotogales. The tree topology is based on the ARB database of 11,800 sequence entries and was reconstructed using the ARB parsimony tool. A filter defining positions that share identical residues in at least 50% of all included sequences from the Thermotogales was used for reconstructing the tree. Reference sequences were chosen to represent the broadest diversity of bacteria. Accession numbers for the sequences are given in Table 1. The scale bar represents 0.10 fixed mutations per nucleotide position.

of the Friedrichstollen in Baden-Baden (Germany; Fig. 3).

Evidence for the presence of Thermotogales relatives in deep-subsurface petroleum reservoirs was reported for the first time by Stetter et al. (1993). *Thermotoga*-related organisms (designated "Pb-isolates") were obtained from seawater flooded oil fields, about 3000 m below the permafrost surface of the North Slope of Alaska (Prudhoe Bay, Endicott and Kuparuk oil fields; Stetter et al., 1993; Stetter and Huber, 2000). By 16S rRNA gene sequence comparison and sequence signature analysis, these Pb-isolates were found to group together with the recently described *T. petrophila* and *T. naphthophila* from the Kubiki oil reservoir in Niigata (Japan; Takahata et al., 2001). Further *Thermotoga* isolates from oil production wells were described as *Thermotoga elfii* (Africa; Ravot et al., 1995), *Thermotoga subterranea* (East Paris Basin; Jeanthon et al., 1995) and *Thermotoga hypogea* (Africa; Fardeau et al., 1997). *Thermotoga*-like organisms have been also reported from a continental oil reservoir (L'Haridon et al., 1995), and Thermotogales relatives were also found during a systematic survey in high temperature oil reservoirs with different salinities (Grassia et al., 1996).

Members of the genus *Fervidobacterium* seem to be restricted to biotopes with low salinity. *Fervidobacterium nodosum* was originally isolated from a hot spring in New Zealand (Patel et al., 1985a). *Fervidobacterium islandicum* was obtained from a continental solfatara field at Hveragerthi (Iceland; Huber et al., 1990) and further, so far undescribed *Fervidobacterium* relatives were isolated from continental hot springs in Tibet (Huber and Stetter, 1992a), from Nadi, Viti Levu (Fiji Island), and from Octopus Spring (Yellowstone National Park, United States). *Fer-*

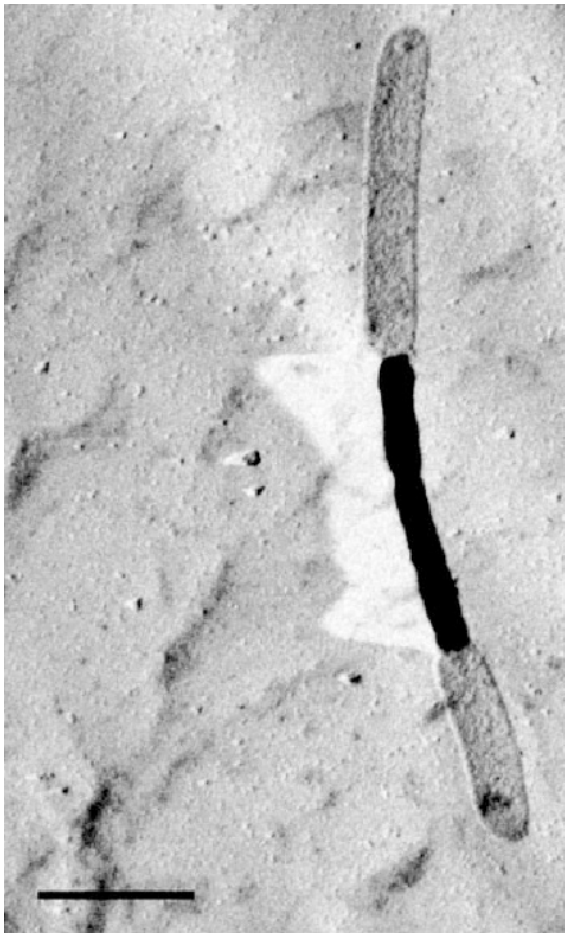

Fig. 3. The new *Thermotoga* species BB13-1-L6A, isolated from geothermally heated water from the Friedrichstollen in Baden-Baden, Germany. The rod-shaped cell is covered by an elongated sheath-like outer membrane ("toga"), overballooning at both ends. Bar, 1 μm.

vidobacterium gondwanense was obtained from a sample taken from a runoff channel of the Great Artesian Basin of Australia (Andrews and Patel, 1996), *Fervidobacterium pennivorans* from a hot spring on San Miguel (the Azores, Portugal; Friedrich and Antranikian, 1996).

From marine hydrothermal springs and sandy sediments of the Gulf of Tadjoura southwest of Obock (Djibouti, Africa), three strains belonging to the genus *Thermosipho* have been isolated. They represented a single species, *Thermosipho africanus* (Huber et al., 1989a). *Thermosipho melanesiensis*, the second species within this genus, was obtained from the gills of *Bathymodiolus brevior*, a deep-sea hydrothermal vent mussel. This invertebrate was collected at the bottom of a black smoker in the Lau Basin (depth 1832 m), Southwest Pacific Ocean (Antoine et al., 1997). Recently, *Thermosipho japonicus* was isolated from a deep-sea hydrothermal vent in the Iheya Basin (Japan; Takai and Horikoshi, 2000a). Members of the genus *Thermosipho* thrive also in hot oil reservoirs. A so far undescribed, new *Thermosipho* species was obtained from reservoir fluids taken at the Thistle oil production platform (East Shetland Basin, North Sea; Stetter et al., 1993; Stetter and Huber, 2000), and recently, *Thermosipho geolei* from a continental petroleum reservoir in Western Siberia was published (Russia; L'Haridon et al., 2001).

From petroleum reservoirs located in Oklahoma and Texas, members of *Geotoga* and *Petrotoga* have been isolated for the first time (Davey et al., 1993a). The isolates have been described as *Geotoga petraea*, *Geotoga subterranea* and *Petrotoga miotherma*. *Petrotoga mobilis* was obtained from a North Sea oil production well (Lien et al., 1998) and two recent isolates were obtained from a continental petroleum reservoir in Western Siberia, namely *Petrotoga olearia* and *Petrotoga sibirica* (L'Haridon et al., 2002).

The type species of *Marinitoga*, *Marinitoga camini*, was isolated from a deep-sea hydrothermal chimney sample on the Mid-Atlantic Ridge (Wery et al., 2001) and *Marinitoga piezophila* was isolated from a chimney rock on the East-Pacific Rise (Alain et al., 2002).

Isolation

Members of the Thermotogales can be enriched in anaerobic culture media with a pH around 7 (Table 2) under a gas phase consisting of 300 kPa N_2 or 300 kPa N_2/CO_2 (80:20; vol/vol). Depending on the salt requirement of the organism, either half strength seawater media or media with low ionic strength are used (Table 2). The media, supplemented with a defined carbon source (e.g., maltose or starch; Table 3) and complex organic material (e.g., yeast extract or peptone; Table 3), are inoculated with original sampling material. Members of the *T. maritima NT. neapolitana* group can be selectively enriched at an incubation temperature of 85°C with starch as the only carbon and energy source. Keratinophilic (feather-degrading) *Fervidobacterium* species might be obtained in yeast extract/tryptone medium with native feathers from chickens, ducks or geese (Leuschner and Antranikian, 1994). For enrichment of *G. petraea*, *G. subterranea* and *P. miotherma*, alpha-glycerophosphate and starch should be added to petroleum reservoir brines (Davey et al., 1993a). *Marinitoga piezophila* can be selectively enriched heterotrophically under a hydrostatic pressure of 30 MPa (Alain et al., 2002).

The enrichment bottles are incubated at the appropriate temperatures (45–85°C; Table 2)

and monitored for growth over a certain time period (e.g., two weeks) by phase contrast microscopy. When rod-shaped cells with an outer sheath-like structure or with a single terminal bleb become visible, the enrichments are serially diluted. The isolates are obtained by plating under anaerobic conditions and at an incubation temperature between 45 and 80°C (Table 2). For plating, a stainless steel anaerobic jar (Balch et al., 1979) and plates, solidified with Gelrite (0.7–1.0%; Huber and Stetter, 2001a) or agar (0.8–3%), can be used. Other methods for isolation are agar shake tubes (2%) or phytagel roll tubes (4%). For *T. neapolitana*, mean plating efficiencies up to 84% on 0.7% Gelrite have been reported. For growth, the plates were incubated at 77°C in a glass canning jar containing a packet of palladium pellets as a catalyst (Childers et al., 1992; Vargas and Noll, 1994). Colonies of the Thermotogales are uniformly round with a diameter of about 1–2 mm. Colonies of the genera *Thermotoga*, *Fervidobacterium*, *Geotoga* and *Petrotoga* appear whitish, while colonies of *Thermosipho* are colorless or brownish.

From liquid cultures, Thermotogales relatives can be also isolated by a plating-independent, newly developed isolation procedure (selected cell cultivation technique) based on optical trapping of single cells using a strongly focused infrared laser beam ("optical tweezers"; Huber et al., 1995; Beck and Huber, 1997; Huber, 1999; Huber and Stetter, 2001a).

In the future, a new isolation strategy might be used to isolate specifically Thermotogales relatives from their biotopes (Huber et al., 1995; Huber et al., 2000). This method combines 16S

Table 2. Physiological properties of the described species within the Thermotogales.

Species	G+C content (mol%)	Temperature optimum (°C)	Temperature range (°C)	pH optimum	pH range	NaCl optimum (%)	NaCl range (%)
Thermotoga maritima	46	80	55–90	6.5	5.5–9.0	2.7	0.25–6.0
Thermotoga neapolitana	41	80	55–90	7.0	5.5–9.0	2.0	0.25–6.0
Thermotoga thermarum	40	70	55–84	7.0	6.0–9.0	0.35	0.2–0.55
Thermotoga elfii	40	66	50–72	7.5	5.5–8.7	1.0	0–2.4
Thermotoga subterranea	40	70	50–75	7.0	6.0–8.5	1.2	0–2.4
Thermotoga hypogea	50	70	56–90	7.3–7.4	6.1–9.1	0.02	0–0.5
Thermotoga petrophila	46.6	80	47–88	7.0	5.2–9.0	1.0	0–5.5
Thermotoga naphthophila	46.1	80	48–86	7.0	5.4–9.0	1.0	0.1–6.0
Thermotoga lettingae	39.2	65	50–75	7.0	6.0–8.5	1.0	0–2.8
Thermosipho africanus	30	75	35–77	7.2	6.0–8.0	n.d.	0.11–3.6
Thermosipho melanesiensis	30.5	70	50–75	6.5–7.5	4.5–8.5	3.0	1.0–6.0
Thermosipho japonicus	31	72	45–80	7.2–7.6	5.3–9.3	4.0	0.66–7.9
Thermosipho geolei	30.0	70	45–75	7.5	6.0–9.4	2–3	0.5–7.0
Fervidobacterium nodosum	33.7	70	41–79	7.0	6.0–8.0	<1.0	n.d.
Fervidobacterium islandicum	40	65	50–80	7.0	6.0–8.0	<0.7	n.d.
Fervidobacterium gondwanense	35	65–68	45–80	7.0	6.0–8.0	<0.2	n.d.
Fervidobacterium pennivorans	40	70	50–80	6.5	5.5–8.0	0.4	0–4
Geotoga petraea	30	50	30–55	6.5	5.5–9.0	3	0.5–10
Geotoga subterranea	30	45	30–66	6.5	5.5–9.0	4	0.5–10
Petrotoga miotherma	40	55	35–65	6.5	5.5–9.0	2	0.5–10
Petrotoga mobilis	31–34	58–60	40–65	6.5–7.0	5.5–8.5	3–4	0.5–9.0
Petrotoga olearia	35	55	37–60	7.5	6.5–8.5	2	0.5–8
Petrotoga sibirica	33	55	37–55	8	6.5–9.4	1	0.5–7.0
Marinitoga camini	29	55	25–65	7.0	5.0–9.0	3.0	1.0–4.5
Marinitoga piezophila	29 (±1)	65	45–70	6.0	5.0–8.0	3.0	1.0–5.0

Abbreviation: n.d., not determined.

Table 3. Substrate specificity of the Thermotogales type strains.

Growth on different substrates

Substrate	Monosaccharide[a]								Di- and trisaccharide[a]				
Species	Arabinose	Fructose	Galactose	Glucose	Mannose	Rhamnose	Ribose	Xylose	Cellobiose	Lactose	Maltose	Sucrose	Raffinose
Thermotoga maritima	nr	+	+	+	nr	nr	+	+	nr	+	+	+	–
Thermotoga neapolitana	nr	+	+	+	nr	nr	+	+	nr	+	+	+	+
Thermotoga thermarum	–	–	–	+	–	nr	nr	+	nr	–	+	+	nr
Thermotoga elfii	+	+	nr	+	+	–	+	+	nd	+	+	+	nr
Thermotoga subterranea	nr	nr	nr	+	nr	nr	–	nr	nr	–	+	nr	–
Thermotoga hypogea	–	+	+	+	+	nr	–	+	nr	+	+	+	nr
Thermotoga petrophila	nr	+	+	+	nr	nr	+	–	nr	+	+	+	nr
Thermotoga naphthophila	nr	+	+	+	nr	nr	+	–	nr	+	+	+	nr
Thermotoga lettingae	+	+	+	+	+	+	+	+	+	+	+	+	nr
Geotoga petraea	nr	nr	+	+	+	nr	nr	–	nr	+	+	+	–
Geotoga subterranea	nr	nr	+	+	+	nr	nr	–	nr	+	+	+	–
Petrotoga miotherma	nr	nr	+	+	+	nr	nr	+	nr	+	+	+	–
Petrotoga mobilis	+	+	+	+	–	+	+	+	+	+	+	+	–
Petrotoga olearia	+	+	nr	+	nr	–	+	+	+	nr	+	+	nr
Petrotoga sibirica	nr	+	+	+	nr	–	+	nr	nr	nr	+	+	nr
Thermosipho africanus	nr	nr	nr	–	nr	nr	–	nr	nr	–	–	nr	–
Thermosipho melanesiensis	nr	–	+	+	nr	nr	nr	–	+	+	+	+	nr
Thermosipho japonicus	nr	nr	+	+	nr	nr	+	nr	nr	nr	+	+	nr
Thermosipho geolei	–	–	–	+	nr	nr	–	–	nr	nr	–	–	nr
Fervidobacterium nodosum	+	+	+	+	+	(+)	–	–	nr	+	+	+	+
Fervidobacterium islandicum	+	+	+	+	+	nr	+	–	nr	–	+	nr	+
Fervidobacterium gondwanense	–	+	+	+	+	nr	–	+	+	+	+	nr	–
Fervidobacterium pennivorans	–	+	+	+	+	nr	+	+	nr	–	+	nr	–
Marinitoga camini	–	+	–	+	–	nr	–	–	+	–	+	+	nr
Marinitoga piezophila	nr	(+)	(+)	(+)	nr	nr	(+)	nr	(+)	nr	(+)	nr	nr

Substrate	Polysaccharide[a]							Alcohol[a]					
Species	Cellulose	Chitin	Glycogen	Maltodextrin	Pectin	Starch	Xylan	Buthanol	Ethanol	Glycerol	Mannitol	Propanol	Sorbitol
Thermotoga maritima	+	nr	+	nr	nr	+	+	nr	–	–	–	nr	nr
Thermotoga neapolitana	+	nr	+	nr	nr	+	nr	nr	–	–	–	nr	nr
Thermotoga thermarum	nr	nr	nr	nr	nr	+	nr	nr	nr	nr	nr	nr	nr
Thermotoga elfii	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	–	nr	nr
Thermotoga subterranea	nr	nr	–	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Thermotoga hypogea	nr	nr	nr	nr	nr	nr	+	nr	nr	nr	nr	nr	nr
Thermotoga petrophila	(+)	–	nr	nr	nr	+	nr	nr	–	nr	–	nr	nr
Thermotoga naphthophila	–	–	nr	nr	nr	+	nr	nr	–	nr	+	nr	nr
Thermotoga lettingae	nr	nr	nr	nr	+	+	+	–	nr	+	–	–	nr
Geotoga petraea	nr	nr	nr	+	nr	+	nr	nr	–	nr	nr	nr	nr
Geotoga subterranea	nr	nr	nr	+	nr	+	nr	nr	–	nr	nr	nr	nr
Petrotoga miotherma	nr	nr	nr	+	nr	+	–	nr	–	nr	nr	nr	nr
Petrotoga mobilis	–	nr	nr	+	nr	+	+	–	–	nr	–	–	nr
Petrotoga olearia	–	nr	nr	nr	nr	+	+	nr	–	nr	–	nr	nr
Petrotoga sibirica	–	nr	nr	nr	nr	nr	+	nr	–	nr	–	nr	nr
Thermosipho africanus	nr	nr	–	nr	nr	nr	nr	nr	–	nr	nr	nr	nr
Thermosipho melanesiensis	nr	nr	–	nr	nr	+	nr	nr	–	nr	–	nr	–
Thermosipho japonicus	nr	–	nr	nr	–	+	–	nr	nr	nr	nr	nr	nr
Thermosipho geolei	–	nr	nr	nr	nr	–	–	nr	nr	nr	nr	nr	nr
Fervidobacterium nodosum	–	nr	nr	nr	+	+	nr	nr	nr	+	nr	nr	+
Fervidobacterium islandicum	+	nr	nr	nr	nr	+	nr	nr	nr	nr	nr	nr	nr
Fervidobacterium gondwanense	–	–	nr	nr	nr	+	–	nr	nr	nr	nr	nr	nr
Fervidobacterium pennivorans	–	nr	+	nr	nr	+	nr	nr	nr	nr	nr	nr	nr
Marinitoga camini	+	+	nr	nr	+	+	–	nr	–	–	nr	nr	nr
Marinitoga piezophila	nr	nr	nr	nr	nr	(+)	nr	nr	nr	nr	nr	nr	nr

Substrate	Organic acid[a]							Complex organic substrate							
Species	Acetate	Butyrate	Lactate	Formate	Propionate	Pyruvate	Succinate	Casein	Casoamino acids	Keratin	Peptone	Tryptone	Yeast extract	Bio-Trypcase	Brain heart infusion
Thermotoga maritima	–	nr	–	–	–	–	nr	nr	–	nr	+	nr	+	nr	nr
Thermotoga neapolitana	–	nr	–	–	–	–	nr	nr	–	nr	(+)	(+)	+	nr	nr
Thermotoga thermarum	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr	nr
Thermotoga elfii	–	–	–	nr	–	nr	nr	nr	nr	nr	nr	nr	nr	+	nr
Thermotoga subterranea	–	nr	–	nr	nr	nr	nr	+	nr	nr	+	+	+	nr	nr
Thermotoga hypogea	–	–	–	nr	–	nr	nr	nr	nr	nr	nr	nr	+	+	nr
Thermotoga petrophila	–	nr	–	–	–	nr	nr	–	–	nr	+	nr	+	nr	nr
Thermotoga naphthophila	–	nr	–	–	–	nr	nr	–	–	nr	+	nr	+	nr	nr
Thermotoga lettingae	+	nr	+	+[1]	nr	+	–	nr	+	nr	+	nr	+	+	nr
Geotoga petraea	–	nr	–	–	nr	nr	nr	nr	–	nr	nr	nr	nr	nr	nr
Geotoga subterranea	–	nr	–	–	nr	nr	nr	nr	–	nr	nr	nr	nr	nr	nr
Petrotoga miotherma	–	nr	–	–	nr	nr	nr	nr	–	nr	nr	nr	nr	nr	nr
Petrotoga mobilis	–	nr	–	–	nr	–	nr	nr	–	nr	nr	nr	nr	nr	nr
Petrotoga olearia	–	nr	nr	–	nr	+	nr	–	–	nr	+	nr	nr	nr	nr
Petrotoga sibirica	–	nr	nr	–	nr	+	nr	–	–	nr	+	nr	nr	nr	nr
Thermosipho africanus	–	nr	–	nr	–	nr	nr	nr	nr	nr	+	+	+	nr	nr
Thermosipho melanesiensis	–	nr	nr	–	nr	nr	nr	nr	–	nr	nr	+	(+)	nr	(+)
Thermosipho japonicus	nr	nr	nr	nr	nr	nr	nr	+	–	nr	+	+	+	nr	nr
Thermosipho geolei	–	nr	nr	–	nr	–	–	–	nr	nr	+	nr	+	nr	nr
Fervidobacterium nodosum	–	nr	–	–	–	(+)	–	nr	nr	nr	nr	nr	nr	nr	nr
Fervidobacterium islandicum	nr	nr	nr	nr	nr	+	nr	nr	nr	nr	nr	nr	nr	nr	nr
Fervidobacterium gondwanense	nr	nr	nr	nr	nr	+	nr	nr	–	nr	nr	nr	nr	nr	nr
Fervidobacterium pennivorans	nr	nr	nr	nr	nr	–	–	–	nr	+	nr	nr	nr	nr	nr
Marinitoga camini	–	nr	–	–	–	+	–	nr	–	–	+	+	nr	nr	nr
Marinitoga piezophila	(+)	nr	nr	nr	nr	nr	nr	+	+	nr	+	+	+	nr	+

Symbols and abbreviations: +, growth; (+), weak or very weak growth; +[1], growth with thiosulfate and H_2/CO_2; –, no growth; and nr, not reported.

[a]For degradation, yeast extract is stimulatory or obligately required.

rRNA gene sequence analysis in situ, specific whole-cell hybridization within enrichments, and the cultivation of cells selected by the use of the laser microscope (Huber et al., 1995).

Identification

Cells of Thermotogales representatives are rod-shaped, about 1.0–50.0 µm long and 0.4–1.0 μm in diameter. They form an outer sheath, ballooning over the ends ('toga'), visible in all phases of growth (Figs. 4 and 5). Usually, they grow singly and in pairs (Fig. 4); members of the genus *Thermosipho* form short chains of up to 12 individuals surrounded by a sheath (Fig. 6). The majority of the *Fervidobacterium* cells form a characteristic terminal bleb ('spheroids') on one end of the cells, which occurs during all stages of growth (diameter 1.0–4.0 µm; Fig. 7). Furthermore, growth in short chains is observed, and *F. islandicum* frequently forms aggregates of up to 50 cells. Besides spheroids, *Fervidobacterium* species form spheres (diameter 5.0–8.0 μm; Fig. 8), membrane-bound structures containing one to seven individual cells. All members of the Thermotogales exhibit a Gram-negative staining reaction, but diamino-pimelic acid, typical for Gram-negative bacteria, is absent.

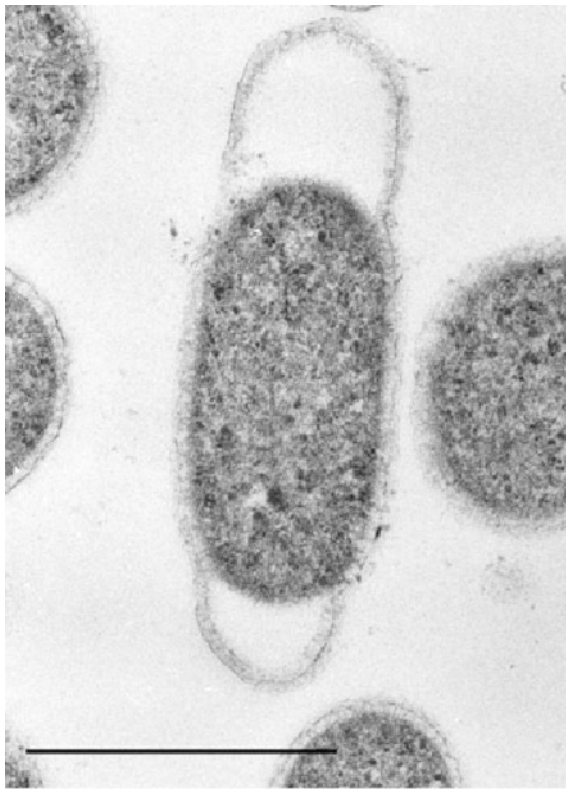

Fig. 5. Ultrathin section of *Thermotoga maritima*. Bar, 1 µm.

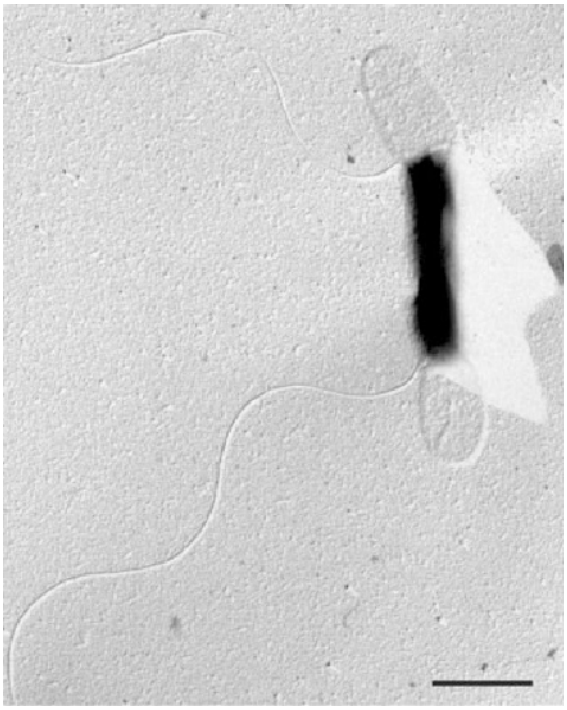

Fig. 4. Platinum-shadowed, flagellated cell of *Thermotoga maritima*. Bar, 1 µm.

Fig. 6. Four rod-shaped cells of *Thermosipho africanus* in a tube-like sheath; platinum-shadowed. Bar, 1 µm.

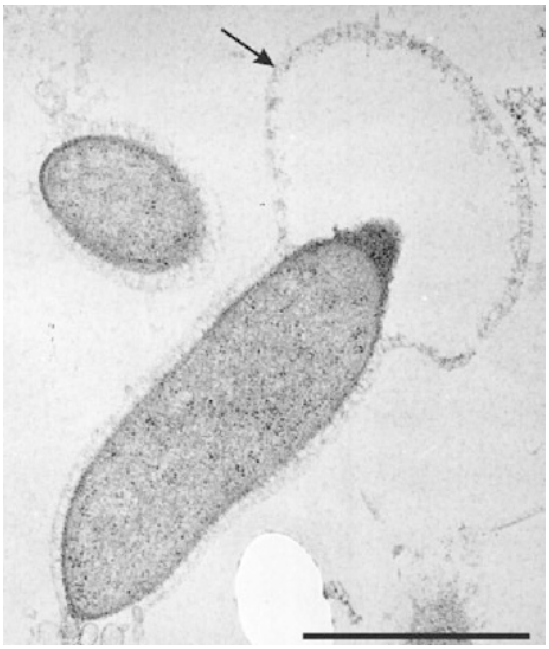

Fig. 7. Ultrathin section of *Fervidobacterium islandicum*. Bar, 1 µm.

Fig. 9. Free-floating biofilm of *Thermotoga* sp. SG1-L2A. Bar, 0.5 cm.

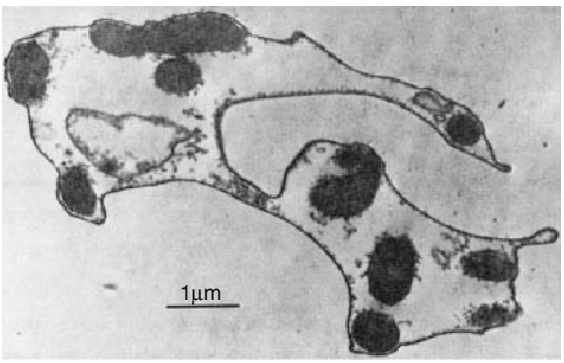

Fig. 8. Ultrathin section of *Fervidobacterium nodosum*. Single sphere, containing several cells. Bar, 1 µm. (Courtesy of H. W. Morgan.)

Thermotogales species either are immotile or possess flagella and are motile (Huber and Stetter, 1999; Huber and Stetter, 2001b; Fig. 4). Motility of *T. maritima* was observed at 60–90°C after heating the microscopic slide. At the optimal growth temperature of 80°C, the maximum speed was approximately 3 mm/min. Furthermore, a thermotactic response to temporal temperature changes was reported for *T. maritima* and thermostable chemotaxis proteins have been identified (Gluch et al., 1995; Swanson et al., 1996). Recently, the crystal structure of CheA, a signal-transducing histidine kinase, and the crystal structures of the middle and C-terminal domain of the flagellar rotor protein FliG from *T. maritima* have been published (Bilwes et al., 1999; Brown et al., 2002).

So far, there is only one report of film formation within the Thermotogales. During continuous cultivation of *T. maritima*, exopolysaccharides were produced under optimal growth conditions. Concomitantly, a thin and white, but not further specified film below the liquid level of the medium was observed (Rinker and Kelly, 2000). Very recently, we found biofilm formation by *Thermotoga* sp. SG1-L2A, produced under physiological growth conditions in batch cultures at the end of the logarithmic growth phase. The cells formed free-floating, white-colored biofilms in the medium with a length of up to 1 cm (Fig. 9).

The ultrastructure of *T. maritima* (Huber et al., 1986) and related species, as determined by transmission electron microscopy by freeze-etching and in ultrathin sections, can only be interpreted as *Thermotoga* being a true Gram-negative bacterium (Rachel et al., 1990). A sheath-like outer membrane and overballooning ends give the cells their characteristic appearance. The porin of the outer membrane has been purified and its specific functional properties characterized after insertion into artificial lipid bilayers (Engel et al., 1993); in addition, its secondary structure contains predominantly beta-sheets and the amino acid composition is characteristic for porins (for a review, see Schirmer,

1998). Outer membrane protein (Omp) alpha is a rod-shaped spacer that spans the periplasm, connecting the outer membrane to the inner body (Engel et al., 1992; Lupas et al., 1995). So far, it is not clear how Omp alpha is connected to the peptidoglycan or the cytoplasmic membrane. It is likely that the COOH-terminal hydrophobic tail of the protein is associated with the porin layer in the "balloon" portion of the cell wall. *Thermotoga* cells have an unusual thin and labile murein layer, presumably owing to a low degree of cross-linking; it is not synthesized in the presence of penicillin G or ampicillin and is sensitive to lysozyme. After addition of lysozyme, the rod-shaped cells of *T. maritima* round up within some minutes, which was also observed for *Ts. africanus* and *F. islandicum*. The murein of *T. maritima* consists of muramic acid, *N*-acetylglucosamine, glutamic acid, alanine and lysine (molar ratio = 0.41:0.69:1.00:1.43:0.89). D- and L-Lysine are present and not found so far in Gram-negative bacteria.

Recently, the crystal structure of the cell division protein FtsA and of the cell division inhibitor MinC was reported from *T. maritima* (van den Ent and Löwe, 2000; Cordell et al., 2001). Biochemical and structural investigations gave evidence that the *T. maritima* MreB protein is the bacterial homolog to the physiological polymer of the eucaryotic F-actin (van den Ent et al., 2001).

Electron microscopic studies showed that the cell envelope of *Fervidobacterium* is composed of two layers. The outer layer protrudes to form spheres (Patel et al., 1985a). In *F. nodosum*, the two layers have an irregular convoluted structure and are connected with regular junctions, and the outer layer is susceptible to lysis by SDS. The cells are able to grow and multiply within the spheres (Patel et al., 1985a).

About 50% of the total polar lipids of *T. maritima* are two amphipathic monopolar glycolipids with a very rare alpha-(1-4) diglucosyl structure (Manca et al., 1992). Unusual long-chain dicarboxylic fatty acids are present in the core lipids of the Thermotogales (Huber et al., 1986; Huber et al., 1989a; Huber et al., 1990; Jeanthon et al., 1995). In *T. maritima*, a total of 37 different fatty acids, including the novel 13,14-dimethyloctacosanedioic acid (Carballeira et al., 1997) and a new ether core lipid, 15,16-dimethyl-30-glyceroloxytriacontanoic acid (DeRosa et al., 1988), have been identified. Comparative analysis showed that this core lipid is characteristic for the genus *Thermotoga* (Huber et al., 1986; DeRosa et al., 1988; Windberger et al., 1989; Jeanthon et al., 1995; Fig. 10).

Members of the Thermotogales differ in their sensitivity to antibiotics (Table 4). For *T. elfii* and *F. pennivorans*, no information on antibiotic sensitivity was reported. Thermotogales representatives are significantly different in their sensitivity to the antibiotic rifampicin, which blocks transcription initiation (Huber and Stetter, 1992a; Table 4). Growth of some species is inhibited by only 1 μg/ml (e.g., *T. thermarum*), while other species (e.g., *T. maritima* and *T. neapolitana*) are resistant even against 100 μg/ml. Unusual for bacteria, the purified RNA polymerase of *T. maritima* is resistant to rifampicin (1 μg/ml), and only 80% of its activity is inhibited by 200 μg/ml (Huber et al., 1986). Growth of *T. maritima* is also not inhibited by 10 μg/ml of the aminoglycoside antibiotics paromomycin, neomycin, streptomycin, gentamicin and kanamycin (Huber et al., 1986). Unprecedented for bacteria, the purified ribosomes of *T. maritima* are resistant to these antibiotics, too (Londei et al., 1988). Another unusual feature of *T. maritima* is the resistance of the elongation factor EF-G to fusidic acid (Huber and Stetter, 1992a).

Cultivation

Owing to the anaerobic nature of the Thermotogales, anaerobic media must be prepared for their cultivation. First, the medium (e.g., 1 liter volume) is flushed with nitrogen for about 20 min to get rid of oxygen. Afterwards, the medium is chemically reduced by the addition of unsterile sodium sulfide and/or cysteine-HCl or after dispension in small portions (see Culture Media) from a sterile, anaerobic stock (see

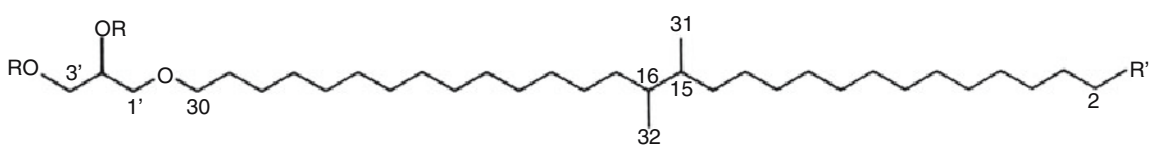

Fig. 10. The new 15,16-dimethyl-30 glyceryloxytriacontanoic acid from *Thermotoga maritima* (courtesy A. Gambacorta).

Table 4. Influence of antibiotics on growth of the Thermotogales species.

Antibiotic (μg/ml)	Ampicillin			Chloramphenicol			Cycloserine		Fusidic acid	Gentamycin		Hygromycin	Kanamycin		Nalidixic acid	Neomycin	Novobiocin	Paromomycin		Penicillin G		Phosphomycin		Polomyxin B	Rifampicin				Spectinomycin	Streptomycin			Tetracyclin	Vancomycin			
Species	10	100	150	10	25	100	10	100	10	10	200	10	10	100	100	10	10	10	10	25	100	10	100	100	1	10	25	100	150	10	25	100	10	10	25	50	100
Thermotoga maritima	n	–	n	n	n	–	n	–	n	n	n	n	n	n	n	n	n	n	n	n	–	n	–	n	n	n	n	+	n	n	n	n	n	n	n	n	–
Thermotoga neapolitana	n	n	n	n	n	–	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	+	n	n	n	–	n	n	n	n	–
Thermotoga thermarum	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	–	n	n	–	n	n	n	n	n	n	n	n	n
Thermotoga elfii	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n
Thermotoga subterranea	n	n	n	n	n	–	n	n	n	n	n	n	n	n	n	n	n	n	n	n	+	n	n	n	n	n	n	+	n	n	n	–	n	n	n	n	n
Thermotoga hypogea	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	+	n	n	n	n	n	n	n	n	n
Thermotoga petrophila	n	n	n	n	n	–	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	–	n	n	n	–	n	n	n	n	–
Thermotoga naphthophila	n	n	n	n	n	–	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	–	n	n	n	–	n	n	n	n	–
Thermotoga lettingae	n	n	n	n	n	–	n	n	n	n	n	n	n	n	n	n	n	n	n	n	+	n	n	n	n	n	n	+	n	n	n	–	n	n	n	n	n
Thermosipho africanus	n	n	n	–	n	–	n	n	n	(–)	n	(–)	(–)	n	n	(–)	n	(–)	n	n	n	–	n	n	n	+	n	–	n	–	n	n	n	–	n	n	–
Thermosipho melanesiensis	n	n	n	–	n	–	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	–	n	–	n	n	n	–	n	n	n	(+)
Thermosipho japonicus	n	n	n	–	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	+	–	n	–	n	n	n	–	n	n	n
Thermosipho geolei	n	n	n	–	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	+	n	–	n	n	n	n	n	n	n	n	+
Fervidobacterium islandicum	–	n	n	–	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	–	n	n	n	–	n	n	n	–	n	n	n
Fervidobacterium nodosum	n	n	n	+	n	n	+	+	n	n	n	n	n	n	n	+	n	n	+	n	n	n	n	n	n	n	n	n	n	+	n	n	–	n	n	n	n
Fervidobacterium gondwanense	n	n	n	n	n	+	n	n	n	n	n	n	n	n	n	n	+	n	+	n	n	n	+	n	+	n	n	n	+	n	+	n	+	+	n	n	n
Fervidobacterium pennivorans	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n
Geotoga petraea	n	n	n	n	n	–	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	–	n	n	n	+	n	n	n	n	–
Geotoga subterranea	n	n	n	n	n	–	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	–	n	n	n	+	n	n	n	n	–
Petrotoga miotherma	n	n	n	n	n	–	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	–	n	n	n	+	n	n	n	n	–
Petrotoga mobilis	–	n	n	–	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	–	n	n	n	n	n	–	n	n	n	–	n	n	n	–	n	n	n
Petrotoga olearia	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	+	n	n	n	+	n	n	n	n	n
Petrotoga sibirica	–	n	n	–	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	n	–	n	n	n	–	n	n	n	–	n
Marinitoga camini	n	n	n	n	–	n	n	n	n	n	n	n	n	+	n	n	n	n	n	–	n	n	n	n	n	n	n	–	n	n	–	n	n	n	–	n	n
Marinitoga piezophila	n	n	–	–	n	n	n	n	–	n	+	n	n	+	–	n	n	n	n	–	n	n	n	n	n	n	–	n	–	n	–	n	–	n	n	n	n

Symbols and abbreviations: –, growth inhibited; (–), temporary inhibition; (+), slight growth; +, growth not inhibited; and n, not reported.

Culture Media). After pH-adjustment, the media are dispensed in 10–20 ml portions in an anaerobic chamber into the culture bottles. They are closed by rubber stoppers and the gas atmosphere is changed to the desired gas mixture (see Culture Media). Thermotogales relatives grow well using 28-ml serum tubes (borosilicate glass; Schott, Mainz, Germany) or 120-ml soda-lime-silicate bottles (Stute GmbH, Rheinbreitbach, Germany). All media were autoclaved for 20 min at 121°C, and the stock-solutions were filter-sterilized.

At large scale, members of the Thermotogales are routinely grown in batch cultures, using enamel-protected fermentors with an operating volume up to 300 liters (HTE, Bioengineering, Wald, Switzerland; Huber et al., 1986). The inhibitory hydrogen gas produced during growth should be removed by continuously flushing the fermentor with nitrogen or argon gas (Huber et al., 1986; Huber et al., 2000).

Culture Media

MSH-Medium for *T. maritima* and *T. neapolitana*

KH_2PO_4	0.5 g
$(NH_4)_2SO_4$	0.5 g
$NaHCO_3$	0.1 g
Yeast extract (BD Difco™)	0.5 g
Starch	5 g
Trace mineral solution (see Trace Mineral Solution)	10 ml
Resazurin	1 mg
Artificial sea water (see Artificial Sea Water)	250 ml
$Na_2S \cdot 9H_2O$	0.5 g
H_2O, double distilled	700.0 ml

Adjust pH to 7.0 with 25% H_2SO_4 and volume to 1 liter with double-distilled water. The headspace of the culture bottles consisted of N_2 (300 kPa).

Trace Mineral Solution (modified; Balch et al., 1979)

Nitrilotriacetic acid	1.5 g
$MgSO_4 \cdot 7H_2O$	3.0 g
$MnSO_4 \cdot H_2O$	0.5 g
NaCl	1.0 g
$FeSO_4 \cdot 7H_2O$	0.1 g
$CoSO_4$	0.1 g
$CaCl_2 \cdot 2H_2O$	0.1 g
$CuSO_4 \cdot 5H_2O$	0.01 g
$AlK(SO_4)_2$	0.01 g
H_3BO_3	0.01 g
$ZnSO_4$	0.1 g
$Na_2WO_4 \cdot 2H_2O$	0.01 g
$Na_2MoO_4 \cdot 2H_2O$	0.01 g
Na_2SeO_4	0.01 g
$(NH_4)_2Ni(SO_4)_2$	0.2 g
H_2O, double distilled	950.0 ml

Adjust pH of the solution to 6.5 with 5N KOH and volume to 1 liter with double-distilled water. Store at 4°C in the dark.

Artificial Sea Water (Huber et al., 1990)

NaCl	27.7 g
$MgSO_4 \cdot 7H_2O$	7.0 g
$MgCl_2 \cdot 6H_2O$	5.5 g
KCl	0.6 g
NaBr	0.1 g
H_3BO_3	0.03 g
$SrCl_2 \cdot 6H_2O$	0.015 g
KI	0.05 mg
$CaCl_2 \cdot 2H_2O$	1.5 g
H_2O, double distilled	950.0 ml

Adjust pH to 5.5–6.0 and volume to 1 liter with double-distilled water.

LA3 Medium for *T. thermarum*

NaCl	3.46 g
$MgSO_4 \cdot 7H_2O$	0.88 g
$MgCl_2 \cdot 6H_2O$	0.69 g
KH_2PO_4	0.5 g
$CaCl_2$	0.09 g
KCl	0.08 g
NaBr	12.50 mg
H_3BO_3	3.75 mg
$SrCl_2 \cdot 6H_2O$	1.9 mg
KI	0.006 mg
$(NH_4)_2Ni(SO_4)_2$	3 mg
Trace mineral solution (see Trace Mineral Solution)	15 ml
EDTA · Na_4-salt	0.9 g
Starch	5 g
Yeast extract (BD Difco™)	0.5 g
Resazurin	1 mg
$Na_2S \cdot 9H_2O$	0.5 g
H_2O, double distilled	950.0 ml

Adjust pH to 7.0 with 25% H_2SO_4 and volume to 1 liter with double-distilled water. The headspace of the culture bottles consisted of N_2 (300 kPa).

Trace Mineral Solution (Balch et al., 1979)

Nitrilotriacetic acid	1.5 g
$MgSO_4 \cdot 7H_2O$	3.0 g
$MnSO_4 \cdot 2H_2O$	0.5 g
NaCl	1.0 g
$FeSO_4 \cdot 7H_2O$	0.1 g
$CoCl_2$	0.1 g
$CaCl_2 \cdot 2H_2O$	0.1 g
$ZnSO_4$	0.1 g
$CuSO_4 \cdot 5H_2O$	0.01 g
$AlK(SO_4)_2$	0.01 g
H_3BO_3	0.01 g
$Na_2MoO_4 \cdot 2H_2O$	0.01 g
H_2O, double distilled	950.0 ml

Adjust pH of the solution to 6.5 with 5N KOH and the volume to 1 liter with double-distilled water. Store at 4°C in the dark.

MB Medium for *T. elfii*

NH_4Cl	1.0 g
K_2HPO_4	0.3 g
KH_2PO_4	0.3 g
$MgCl_2 \cdot 6H_2O$	0.2 g
$CaCl_2 \cdot 2H_2O$	0.1 g
NaCl	10.0 g
KCl	0.1 g
Sodium acetate	0.5 g

Trace mineral solution (see LA3-medium)	10 ml
Yeast extract (BD Difco™)	2.0 g
Bio-Trypcase (bioMérieux)	2.0 g
Resazurin	1 mg
Cysteine · HCl	0.5 g
$Na_2S \cdot 9H_2O$	0.4 g
Na_2CO_3	2.0 g
H_2O, double distilled	950.0 ml

Dissolve all components except the $Na_2S \cdot 9H_2O$ and Na_2CO_3. Adjust pH to 8.0 with 10M KOH and volume to 1 liter with double-distilled water. Autoclave (121°C, 20 min) the medium, then add the $Na_2S \cdot 9H_2O$ and Na_2CO_3 (from sterile, anaerobic stocks). Store or incubate medium in a gas phase of N_2/CO_2 (80:20 v/v; 200 kPa).

Medium for *T. subterranea*

NaCl	12.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g
PIPES (piperazine-*N*, *N*′-bis[ethanesulfonic acid])	3.4 g
KCl	2.0 g
NH_4Cl	0.1 g
$CaCl_2 \cdot 2H_2O$	25 mg
K_2HPO_4	20 mg
Trace mineral solution (see LA3 medium)	10 ml
Vitamin solution (see Vitamin Solution	10 ml
Yeast extract	0.5 g
Peptone	1.0 g
Resazurin	1 mg
$Na_2S \cdot 9H_2O$	0.5 g
H_2O, double distilled	950.0 ml

Add the $Na_2S \cdot 9H_2O$ from a sterile, anaerobic stock to the autoclaved (121°C, 20 min) solution of all other components. Adjust the pH to 7.0 with 25% H_2SO_4 and the volume to 1 liter with double-distilled water. Store or incubate medium in an atmosphere of N_2 (100 kPa).

Vitamin Solution (Balch et al., 1979)

Biotin	2 mg
Folic acid	2 mg
Pyridoxine · HCl	10 mg
Thiamine · HCl	5 mg
Riboflavin	5 mg
Nicotinic acid	5 mg
DL-Calcium pantothenate	5 mg
Vitamin B_{12}	0.10 mg
p-Aminobenzoic acid	5 mg
Lipoic acid	5 mg
H_2O, double distilled	950.0 ml

Adjust volume to 1 liter with double-distilled water, filter-sterilize, and store in the dark at 4°C.

Modified MB Medium for *T. hypogea*

NaCl	10.0 g
NH_4Cl	1.0 g
K_2HPO_4	0.3 g
KH_2PO_4	0.3 g
$MgCl_2$	0.5 g
$CaCl_2$	0.1 g
KCl	0.2 g
Trace mineral solution (see LA3 Medium)	10 ml
Yeast extract (BD Difco™)	2.0 g
Bio-Trypcase (bioMérieux)	2.0 g
Xylan	10.0 g
Resazurin	1 mg
Na_2CO_3	2.0 g
$Na_2S_2O_3 \cdot 5H_2O$	20 mM
Cysteine · HCl	0.5 g
$Na_2S \cdot 9H_2O$	0.4 g
H_2O, double distilled	950.0 ml

Add the Na_2CO_3, $Na_2S_2O_3 \cdot 5H_2O$, and $Na_2S \cdot 9H_2O$ (from sterile, anaerobic stocks) to the autoclaved (121°C, 20 min) solution of the remaining components. Adjust pH to 8.0 with 10M KOH and the volume to 1 liter with double-distilled water. Store or incubate medium in an atmosphere of N_2/CO_2 (80:20 v/v; 100 kPa).

Modified DSMZ Medium No. 664 for *T. lettingae*

NH_4Cl	1.0 g
K_2HPO_4	0.3 g
KH_2PO_4	0.3 g
$MgCl_2 \cdot 6H_2O$	0.2 g
$CaCl_2 \cdot 2H_2O$	0.1 g
KCl	0.1 g
NaCl	10.0 g
Na_2CO_3	2.0 g
$Na_2S_2O_3 \cdot 5H_2O$	5.0 g
Yeast extract	0.5 g
Glucose	4.0 g
Trace mineral solution (see Trace Mineral Solution [DSMZ Medium 141])	10.0 ml
Resazurin	0.5 mg
Cysteine · HCl	0.5 g
$Na_2S \cdot 9H_2O$	0.4 g
$NaHCO_3$	2.0 g
H_2O, double distilled	950.0 ml

Add the Na_2CO_3, $Na_2S_2O_3 \cdot 5H_2O$, glucose, $Na_2S \cdot 9H_2O$, and $NaHCO_3$ (from a sterile, anaerobic stocks) to an autoclaved (121°C, 20 min) solution of the remaining components. Adjust the pH to 7.0 with 10M KOH and the volume to 1 liter with double-distilled water. Store or incubate medium in an atmosphere of N_2/CO_2 (80:20 v/v; 100 kPa).

Trace Mineral Solution (DSMZ Medium 141)

Nitrilotriacetic acid	1.5 g
$MgSO_4 \cdot 7H_2O$	3.0 g
$MnSO_4 \cdot 2H_2O$	0.5 g
NaCl	1.0 g
$FeSO_4 \cdot 7H_2O$	0.1 g
$CoSO_4 \cdot 7H_2O$	0.18 g
$CaCl_2 \cdot 2H_2O$	0.1 g
$ZnSO_4 \cdot 7H_2O$	0.18 g
$CuSO_4 \cdot 5H_2O$	0.01 g
$AlK(SO_4)_2 \cdot 12H_2O$	0.02 g
H_3BO_3	0.01 g
$Na_2MoO_4 \cdot 2H_2O$	0.01 g
$NiCl_2 \cdot 6H_2O$	0.025 g
$Na_2SeO_3 \cdot 5H_2O$	0.3 g
H_2O, double distilled	950.0 ml

Prior to adding nitrilotriacetic acid to the other solution components, adjust its pH to 6.5 with 5N KOH. Adjust pH to 7.0 with 10 M KOH and volume to 1 liter with double-distilled water.

YE Medium for *T. petrophila* and *T. naphthophila*

Yeast extract	2.0 g
$Na_2S \cdot 9H_2O$	0.4 mM
Artificial sea water (ASW; see Artificial Sea Water)	950.0 ml

Add the $Na_2S \cdot 9H_2O$ (from a sterile, anaerobic stock) to an autoclaved (121°C, 20 min) solution of the remaining components. Adjust pH to 7.0 with 6M HCl and the volume to 1 liter with artificial sea water. Store or incubate medium in an atmosphere of N_2 (100kPa).

Artificial Sea Water (ASW; Jannasch et al., 1995)

NaCl	20.0 g
$MgCl_2 \cdot 6H_2O$	3.0 g
$MgSO_4 \cdot 7H_2O$	6.0 g
$(NH_4)_2SO_4$	1.0 g
$CaCl_2 \cdot 2H_2O$	0.3 g
KH_2PO_4	0.2 g
KCl	0.5 g
NaBr	0.05 g
H_3BO_3	0.025 g
$SrCl_2 \cdot 6H_2O$	0.02 g
Ferric ammonium citrate	0.01 g
Bis-tris-propane	2.25 g
Trace mineral solution (see Trace Mineral Solution)	10 ml
Vitamin solution (see Vitamin Solution)	10 ml
Resazurin	0.6 mg
H_2O, double distilled	950.0 ml

Adjust volume to 1 liter with double-distilled water.

Trace Mineral Solution (Wolin et al., 1963)

Nitrilotriacetic acid	1.5 g
$MgSO_4$	3.0 g
$MnSO_4$	0.5 g
NaCl	1.0 g
$FeSO_4$	0.1 g
$CoCl_2$	0.1 g
$CaCl_2$	0.1 g
$ZnSO_4$	0.1 g
$CuSO_4$	0.01 g
$AlK(SO_4)_2$	0.01 g
H_3BO_3	0.01 g
Na_2MoO_4	0.01 g
H_2O, double distilled	950.0 ml

Adjust volume to 1 liter with double-distilled water.

Vitamin Solution (Bazylinski et al., 1989)

Niacin	10 mg
Calcium pantothenate	10 mg
p-Aminobenzoic acid	10 mg
Thiamine	10 mg
Riboflavin	10 mg
Pyridoxine	10 mg
Cobalamin	10 mg
Thioctic (alpha-lipoic) acid	10 mg
Folic acid	4 mg
Biotin	4 mg
H_2O, double distilled	950.0 ml

Filter sterilize and store solution in the dark at 4°C.

MG Medium for *Ts. africanus*

NaCl	18.0 g
$MgSO_4 \cdot 7H_2O$	3.45 g
$MgCl_2 \cdot 6H_2O$	4.30 g
KCl	0.34 g
NH_4Cl	0.25 g
$CaCl_2 \cdot 2H_2O$	0.14 g
$K_2HPO_4 \cdot 3H_2O$	0.14 g
$(NH_4)_2Fe(SO_4)_2$ (0.2%)	1.0 ml
$(NH_4)_2Ni(SO_4)_2$ (0.2%)	1.0 ml
Sodium acetate $\cdot 3H_2O$	1.0 g
Trace mineral solution (see: MSH Medium)	10 ml
Vitamin solution (see: *T. subterranea* Medium)	10 ml
Yeast extract (BD Difco™)	2.0 g
Peptone (pancreatic digest of casein; Merck) (pankreatisch verdaut verwendet)	2.0 g
Resazurin (0.1%)	0.5 ml
$Na_2S \cdot 9H_2O$	0.5 g
H_2O, double distilled	950.0 ml

Add the trace mineral solution (from a sterile, anaerobic stock) to an autoclaved (121°C, 20 min) solution of the remaining components.Adjust pH to 6.5 with 25% formic acid and volume to 1 liter with double-distilled water. Store or incubate medium in an atmosphere of N_2 (300 kPa).

DSMZ Medium No. 343 for *Ts. melanesiensis*

NaCl	20.0 g
KH_2PO_4	0.5 g
$NiCl_2 \cdot 6H_2O$	2.0 mg
Trace mineral solution (see *T. lettingae* Medium)	15.0 ml
Yeast extract (BD Difco™)	0.5 g
Starch	5.0 g
Resazurin	1.0 mg
$Na_2S \cdot 9H_2O$	0.5 g
Artificial sea water (see: MSH Medium)	250 ml
H_2O, double distilled	700.0 ml

Adjust pH to 6.5 with 25% formic acid and volume to 1 liter with double-distilled water. Store or incubate medium in an atmosphere of N_2 (100 kPa).

MJDYP Medium for *Ts. japonicus*

Yeast extract	2.0 g
Trypticase peptone	2.0 g
Resazurin	1.0 mg
$Na_2S \cdot 9H_2O$	0.5 g
MJD synthetic water (see MJD Synthetic Water)	1000.0 ml

Adjust pH to 7.5 with 6N HCl. Store or incubate medium in an atmosphere of N_2 (200 kPa).

MJD Synthetic Water

NaCl	30.0 g
K_2HPO_4	0.14 g
$CaCl_2 \cdot 2H_2O$	0.14 g
$MgSO_4 \cdot 7H_2O$	3.4 g
$MgCl_2 \cdot 6H_2O$	4.18 g
KCl	0.33 g
$NiCl_2 \cdot 6H_2O$	0.5 mg

$Na_2SeO_3 \cdot 5H_2O$	0.5 mg
$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	0.01 g
Trace mineral solution (see LA3 Medium)	10 ml
DHV mineral solution (see DHV Mineral Solution)	1 ml
H_2O, double distilled	950.0 ml

Adjust volume to 1 liter with double-distilled water.

DHV Mineral Solution

$Na_2SiO_3 \cdot 9H_2O$	2.3 g
$SrCl_2 \cdot 6H_2O$	1.1 g
$CoSO_4 \cdot 7H_2O$	1.1 g
$Na_2MoO_4 \cdot 2H_2O$	0.97 g
$MnSO_4 \cdot 2H_2O$	0.96 g
$NiCl_2 \cdot 6H_2O$	0.95 g
$Na_2WO_4 \cdot 2H_2O$	0.66 g
$ZnSO_4 \cdot 7H_2O$	0.58 g
$CuSO_4 \cdot 5H_2O$	0.50 g
$VSO_4 \cdot xH_2O$	0.25 g
Na_2SeO_3	0.17 g
$LiSO_4 \cdot H_2O$	0.13 g
H_2O, double distilled	950.0 ml

Adjust volume to 1 liter with double-distilled water.

Medium for *Ts. geolei*

NaCl	15.0 g
$MgCl_2 \cdot 6H_2O$	0.5 g
PIPES	3.4 g
KCl	0.2 g
NH_4Cl	1.0 g
$CaCl_2 \cdot 2H_2O$	0.1 g
K_2HPO_4	0.35 g
KH_2PO_4	0.35 g
Sodium acetate $\cdot 3H_2O$	2.72 g
Yeast extract	2.0 g
Bio-Trypcase (bioMérieux)	2.0 g
Resazurin	1.0 mg
Maltose	2.0 g
$Na_2S \cdot 9H_2O$	1.0 g
H_2O, double distilled	950.0 ml

Add the maltose and $Na_2S \cdot 9H_2O$ (from sterile, anaerobic stocks) to an autoclaved (121°C, 20 min) solution of the remaining components. Adjust pH to 7.0 with 5M HCl and volume to 1 liter with double-distilled water. Store or incubate medium in an atmosphere of N_2 (100 kPa).

TYEG Medium for *F. nodosum*, *F. islandicum* and *F. gondwanense*

NH_4Cl	0.9 g
$MgCl_2 \cdot 6H_2O$	0.2 g
KH_2PO_4	0.75 g
K_2HPO_4	1.5 g
$FeSO_4 \cdot 7H_2O$ (10%)	0.03 ml
Trace mineral solution (see Trace Mineral Solution)	9.0 ml
Vitamin solution (see *T. subterranea* Medium)	5.0 ml
Yeast extract (BD Difco™)	3.0 g
Peptone (from tryptically digested meat; Merck)	10.0 g
Glucose	5.0 g
Resazurin (0.2%)	1.0 ml
$Na_2S \cdot 9H_2O$	1.0 g
H_2O, double distilled	950.0 ml

Add the vitamin solution and glucose (from a sterile, anaerobic stock) to an autoclaved (121°C, 20 min) solution of the remaining components. Adjust pH to 7.2 with 25% H_2SO_4 and the volume up to 1 liter with double-distilled water. Store or incubate medium in an atmosphere of N_2 (300 kPa).

Trace Mineral Solution (Patel et al., 1985a)

Nitrilotriacetic acid	12.5 g
$FeCl_3 \cdot 4H_2O$	0.2 g
$MnCl_2 \cdot 4H_2O$	0.1 g
$CoCl_2 \cdot 6H_2O$	0.017 g
$CaCl_2 \cdot 2H_2O$	0.1 g
$ZnCl_2$	0.1 g
$CuCl_2$	0.02 g
H_3BO_3	0.01 g
$Na_2MoO_4 \cdot 2H_2O$	0.01 g
NaCl	1.0 g
Na_2SeO_3	0.02 g
H_2O, double distilled	950.0 ml

Prior to adding to the trace mineral solution, adjust pH of the nitrilotriacetic acid to 6.5 with 5N KOH. Adjust pH of the trace mineral solution to 6.5 with 5N KOH and the volume of the trace mineral solution to 1 liter with double-distilled water. Store medium at 4°C in the dark.

TF Medium for *F. pennivorans*

NH_4Cl	0.50 g
$MgSO_4 \cdot 7H_2O$	0.16 g
K_2HPO_4	1.6 g
$NaH_2PO_4 \cdot H_2O$	1.0 g
$CaCl_2 \cdot 2H_2O$	0.06 g
Trace mineral solution (see: LA3 Medium)	10 ml
Vitamin solution (see *T. subterranea* Medium)	10 ml
Yeast extract	2.0 g
Trypticase	2.0 g
Resazurin	1 mg
$Na_2S \cdot 9H_2O$	0.3 g
Cysteine $\cdot$ HCl	0.3 g
Glucose or starch	3.0 g
H_2O, double distilled	950.0 ml

Add the $CaCl_2 \cdot 2H_2O$ (from a sterile, anaerobic stock) to an autoclaved (121°C, 20 min) solution of the remaining components. Adjust pH to 6.8 with 10N KOH and the volume up to 1 liter with double-distilled water. Store or incubate medium in an atmosphere of N_2.

MSH Medium for *P. miotherma*, *G. petraea* and *G. subterranea*

See MSH Medium for *T. maritima* and *T. neapolitana*

Medium for *P. mobilis*

NaCl	30.0 g
$MgSO_4 \cdot 7H_2O$	7.0 g
KCl	0.34 g
NH_4Cl	0.25 g
$CaCl_2 \cdot 2H_2O$	0.14 g
KH_2PO_4	0.14 g
Yeast extract (BD Difco™)*	0.2 g
Trace element solution SL-10 (see Trace Element Solution SL-10)	1.0 ml
Vitamin solution (see *T. subterranea* Medium)*	10 ml

Resazurin (0.02%)	0.5 ml
$Na_2S \cdot 9H_2O$ (0.5 M)	4 ml
H_2O, double distilled	950.0 ml

Add the yeast extract and the vitamin solution (from a sterile, anaerobic stock) to an autoclaved (121°C, 20 min) solution of the remaining components. Adjust pH to 6.5 with 6M HCl and the volume up to 1 liter with double-distilled water. Store or incubate medium in an atmosphere of argon (200 kPa).

Trace Element Solution SL-10 (Widdel et al., 1983)

HCl (25% w/w, 7.7 M)	10 ml
$FeCl_2 \cdot 4H_2O$	1.5 g
$CoCl_2 \cdot 6H_2O$	190 mg
$MnCl_2 \cdot 4H_2O$	100 mg
$ZnCl_2$	70 mg
H_3BO_3	6 mg
$Na_2MoO_4 \cdot 2H_2O$	36 mg
$NiCl_2 \cdot 6H_2O$	24 mg
$CuCl_2 \cdot 2H_2O$	2 mg
H_2O, double distilled	950.0 ml

Adjust volume up to 1 liter with double-distilled water.

Medium for *P. olearia* and *P. sibirica*

NaCl	25.0 g
$MgCl_2 \cdot 6H_2O$	0.5 g
PIPES	3.4 g
KCl	0.2 g
NH_4Cl	1.0 g
$CaCl_2 \cdot 2H_2O$	0.1 g
K_2HPO_4	0.35 g
KH_2PO_4	0.35 g
Peptone	1.5 g
Tryptone	1.5 g
Yeast extract	1.5 g
Maltose	2.0 g
Resazurin	1.0 mg
$Na_2S \cdot 9H_2O$	1.0 g
H_2O, double distilled	950.0 ml

Add the maltose and $Na_2S \cdot 9H_2O$ (from sterile, anaerobic stocks) to an autoclaved (121°C, 20 min) solution of the remaining components. Adjust pH to 7.0 with 5M HCl and the volume up to 1 liter with double-distilled water. Store or incubate medium in an atmosphere of N_2 (100 kPa).

YPCS Medium for *M. camini*

Yeast extract (BD Difco™)	0.5 g
Peptone (BD Difco™)	1.0 g
Cellobiose (Sigma-Aldrich)	5.0 g
Sea salt (Sigma-Aldrich)	30.0 g
PIPES buffer (Sigma-Aldrich)	6.05 g
Resazurin	1.0 mg
$Na_2S \cdot 9H_2O$	0.5 g
H_2O, double distilled	950.0 ml

Adjust pH to 7.5 with 5M HCl and the volume up to 1 liter with double-distilled water. Store or incubate medium in an atmosphere of $N_2/H_2/CO_2$ (90:5:5 v/v/v, 100 kPa).

RC+ Medium for *M. piezophila*

NaCl	30.0 g
MES (2-[*N*-morpholino]ethanesulfonic acid); Sigma-Aldrich)	10 mM
NH_4Cl	1.0 g
K_2HPO_4	0.3 g
$MgCl_2 \cdot 6H_2O$	0.2 g
$CaCl_2 \cdot 2H_2O$	0.1 g
KCl	0.1 g
Sodium acetate $\cdot 3H_2O$	0.83 g
Yeast extract (BD Difco™)	5.0 g
Bio-Trypcase (BD Difco™)	5.0 g
Maltose (Sigma-Aldrich)	20 mM
L-Cystine	12.0 g
Resazurin	1.0 mg
$Na_2S \cdot 9H_2O$	0.5 g
H_2O, double distilled	950.0 ml

Add the L-cystine and $Na_2S \cdot 9H_2O$ (from sterile, anaerobic stocks) to an autoclaved (121°C, 20 min) solution of the remaining components. Adjust pH to 6.0 with 5M HCl and the volume up to 1 liter with double-distilled water. Store or incubate medium in an atmosphere of $N_2/H_2/CO_2$ (90:5:5 v/v/v, 100 kPa up to 40 MPa).

Preservation

Under anaerobic conditions, members of the Thermotogales can be stored for several months at 4°C. For long-term preservation, storage at –140°C in liquid nitrogen in the presence of 5% dimethylsulfoxide is recommended (Malik, 1999). No loss of cell viability of *Thermotoga*, *Thermosipho* and *Fervidobacterium* was observed after storage over a period of more than ten years. *Thermotoga subterranea* was stored at –80°C in the same medium containing 20% (w/v) glycerol (Jeanthon et al., 1995).

Physiology

Members of the Thermotogales are strictly anaerobic, extremely thermophilic or hyperthermophilic bacteria with optimal growth in the neutral pH range (Huber and Stetter, 1999; Table 2). With an optimal growth temperature around 80°C and a maximum growth temperature of 90°C, the hyperthermophilic species *T. maritima* and *T. neaplitana* represent the organisms with the highest growth temperatures within the order. Growth of some Thermotogales species is restricted to low salinity, while other species exhibit a broad salt tolerance, reflecting their adaptation to the natural biotope (Huber and Stetter, 1992a; Huber and Stetter, 1999; Table 2). Detailed physiological properties of the Thermotogales are listed in Table 2.

All members of the Thermotogales are strict organotrophs, fermenting preferentially simple and complex carbohydrates or complex organic matter (Table 3). When grown on defined carbon sources, the addition of yeast extract and/or peptone/bio-trypticase to the medium is often stimulatory or required for growth (Table 3; see

also Culture Media). In addition, cell homogenates of bacteria (e.g., *Lactobacillus bavaricus*) and archaea (e.g., *Pyrodictium brockii*) can be used as substrates (Huber et al., 1986). Furthermore, *T. lettingae* is able to degrade methanol, but only in the presence of yeast extract, which is required for growth (Balk et al., 2002).

With glucose as growth substrate, Thermotogales representatives form L(+)-lactate, acetate, ethanol, L-alanine, carbon dioxide, and hydrogen as major final products. Some of the isolates form traces of isovaleric acid, isobutyric acid, alpha-aminobutyrate, hydroxy-phenylacetate and phenylacetate in addition. The L-alanine production of members of the Thermotogales is a trait in common with members of the archaeal order Thermococcales. Therefore, it has been proposed that L-alanine production from sugar fermentation is a remnant of an ancestral metabolism (Ravot et al., 1996a). The pathway of arginine synthesis is the only biosynthetic reaction studied in the Thermotogales so far. *Thermotoga maritima* generates arginine from glutamate via *N*-acetylated intermediates in an eight-step pathway, as in mesophilic bacteria (Van de Casteele et al., 1990).

Thermotoga maritima degrades glucose mainly via the Embden-Meyerhof glycolytic pathway and, to a lesser extent, via the Entner-Doudoroff pathway (Schröder et al., 1994; Selig et al., 1997). In *T. neapolitana*, it was shown that D-glucose is taken up via an active transport system, energized by an ion gradient. This gradient is generated by ATP, derived from substrate-level phosphorylation (Galperin et al., 1996). The role of this gradient is not known since glucose (and maltose) periplasmic binding proteins appear to be involved in transport (Nanavati et al., 2002).

Hydrogen, which accumulates during fermentation processes, can be a potent inhibitor of growth of the Thermotogales. This inhibition can be overcome by the addition of sulfur or inorganic sulfur-containing compounds, depending on the species. Under these culture conditions, H_2S is formed as final product. Therefore, H_2S formation may be a kind of detoxification reaction of H_2 (Huber et al., 1986; Huber et al., 1992a). A general trait of different Thermotogales is the production of H_2S when grown in the presence of thiosulfate, except for *Ts. geolei*, *M. piezophila*, *P. olearia* and *P. sibirica* (Ravot et al., 1995; L'Haridon et al., 2001; L'Haridon et al., 2002; Alain et al., 2002). In addition to removal by sulfurous compounds, hydrogen can be removed by gassing the media with nitrogen or argon (Huber et al., 1986; Huber and Stetter, 1999) or by interspecies hydrogen transfer during cocultivation of the Thermotogales with hydrogen-consuming hyperthermophiles (e.g., *Methanococcus*, *Methanopyrus*, *Archaeoglobus* or *Ferroglobus*; Huber et al., 2000).

Recently, respiratory growth of *T. maritima* had been reported, with hydrogen as electron donor and Fe(III) as the electron acceptor, forming Fe(II) as final product (Vargas et al., 1998). In contrast, growth of *T. maritima* with similar doubling times and final cell densities, in the absence of both hydrogen and Fe(III) in the same culture medium, was observed (Huber and Stetter, 2001a). Furthermore, when *T. maritima* was cultivated in the presence of hydrogen (100 kPa) and Fe(III), Fe(II) was formed without obvious growth stimulation (Huber and Stetter, 2001a). These results indicate that *T. maritima* may not gain energy by iron-respiration but may use Fe(III) in place of sulfur as an additional electron sink to get rid of inhibitory hydrogen during fermentation (Huber et al., 1986; Schröder et al., 1994).

Different low-molecular-weight organic compounds, serving as compatible solutes, are present in the different members of the Thermotogales (Martins et al., 1996; Ramakrishnan et al., 1997). However, the accumulation of solutes in response to either salt stress or supraoptimal temperatures is restricted to Thermotogales species that grow in saline media containing over 0.4% sodium chloride (Martins et al., 1996).

An enormous variety of enzymes (and proteins) from the order Thermotogales, especially *T. maritima*, has been isolated and intensively studied since the late 1980s. In general, these enzymes exhibit a high thermostability, with denaturation temperatures often above the optimum growth temperature of the bacterium. Despite the strict anaerobic nature of *T. maritima*, these enzymes are apparently not oxygen sensitive. A comprehensive overview of the biochemistry and biophysics of these "hyperthermophilic enzymes and proteins" was published recently in volumes 330, 331 and 334 of *Methods in Enzymology* (2001).

Genetics

So far, the only plasmids discovered in the Thermotogales are in *Thermotoga* sp. RQ7, an isolate obtained from the hot sea-floor of Ribeira Quente (the Azores), and in *Thermotoga maritima* MC24, an isolate from hydrothermal vents near the Kuril Islands (Huber et al., 1986; Harriott et al., 1994; Akimkina et al., 1999). These small cryptic miniplasmids, pRQ7 and pMC24, are very closely related and were shown to be the smallest natural replicon so far described (846 bp). Both were shown to be negatively supercoiled (Harriott et al., 1994; Akimkina et al., 1999). The plasmid pRQ7 replicates by the

rolling-circle mechanism (Yu and Noll, 1997). With pRQ7, a vector system was developed and a genetic transformation of *T. maritima* and *T. neapolitana* spheroplasts was achieved using cationic liposomes (Yu et al., 2001).

For genetic studies, auxotrophic and antimetabolite-resistant mutants of *T. neapolitana* have been isolated by the use of mutagenic agents (Vargas and Noll, 1994).

The rather small, 1.8-Mb genome from *T. maritima* has been sequenced at The Institute for Genomic Research (TIGR; Rockville, MD, USA; Nelson et al., 1999). It is a single circular chromosome and contains 1877 predicted coding regions. Fifty-four percent of these coding regions have functional assignments, while 46% are of unknown function (Nelson et al., 1999; Nelson et al., 2001). Genome analysis has identified metabolic pathways involved in the degradation of sugar and plant polysaccharides. About 7% of the predicted codon sequences in the genome are involved in the metabolism of simple and complex sugars, a percentage more than twice that seen in the genomes of other bacteria or archaea (Nelson et al., 2001). The metabolism and transport in *T. maritima* (Fig. 11) were elucidated from the genome sequence data.

Comparison of total genome sequences of different organisms showed that about 24% of the *T. maritima* genes are of archaeal origin (Nelson et al., 1999). Therefore, the authors have claimed that hyperthermophilic archaea and bacteria have often exchanged genes, particularly by lateral gene transfer (Aravind et al., 1998; Nelson et al., 1999; see chapter on Aquificales in this Volume). On the other hand, it was argued that if *Thermotoga* (and *Aquifex*; see chapter on Aquificales in this Volume) are truly deep branching, it is equally parsimonious to suppose that some of the genes are primitive features shared by these hyperthermophilic bacteria with archaea. They have retained ancestral genes that have since been lost in most bacteria as they adapted to mesophilic environments (Kyrpides and Olson, 1999; Logsdon and Faguy, 1999). On the basis of recent phylogenetic analyses of archaeal genes in different bacteria, it was hypothesized that lateral gene transfer in hyper-

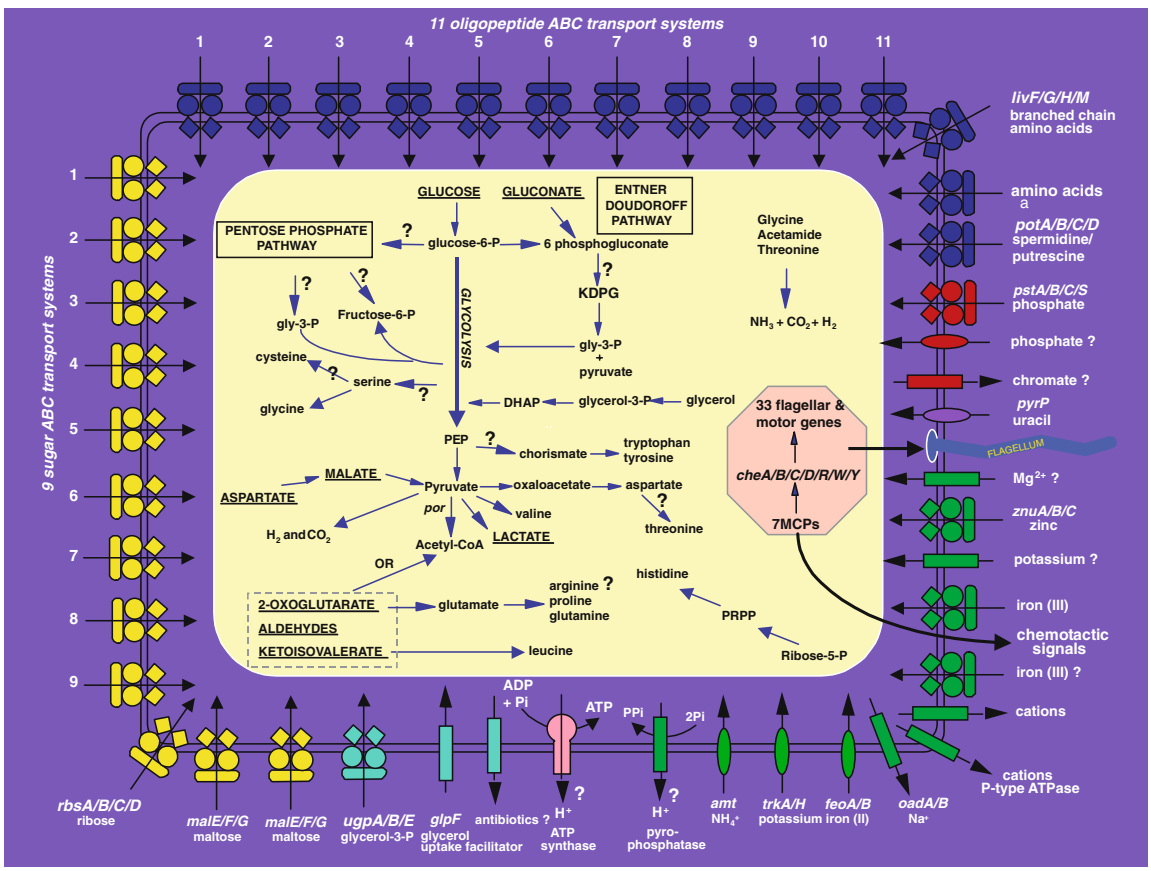

Fig. 11. Overview of metabolism and transport in *Thermotoga maritima*. Pathways for energy production and the metabolism of organic compounds, acids and aldehydes are shown. (From Nelson et al., 1999, with permission from Nature, http://www.nature.com.)

thermophiles may be as much the consequence as the cause of adaptation to hyperthermophily (Nesbø et al., 2001). By using suppressive subtractive hybridization, extensive genomic diversity in *T. maritima* was reported recently and frequent lateral gene transfer within the order Thermotogales was suggested (Nesbø et al., 2002).

Very recently, a high-throughput structural genomics pipeline and its application to the proteome of *T. maritima* were designed and implemented. By using this pipeline, 1376 of the predicted 1877 genes (73%) were successfully cloned, their expression was attempted, and the crystallization conditions for 432 proteins (23% of the *T. maritima* proteome) were identified (Lesley et al., 2002).

Biotechnology

Enzymes active at high temperatures are sought for industrial processes. Intra- and extracellular enzymes of Thermotogales are highly thermostable and might be of great interest as biocatalysts, e.g., in the chemical or food industry. A recombinant xylanase from *T. maritima* was shown to be active for several hours at 100°C and efficient in releasing lignin from kraft pulp (Chen et al., 1997). Therefore, such xylanases have a high potential for use in the pulp and paper industry. Highly thermostable amylases might be used in starch processing and high-temperature glucose isomerases in the production of corn syrup. From *T. maritima*, the recombinant Ultma™ DNA polymerase with proofreading activities is commercially available. The 70-kDa enzyme is highly thermostable and has a half-life of 40–50 min at 97.5°C. In addition, the sequencing of the total genome of *T. maritima* makes available the use of its genes as tools for a variety of biotechnological applications (Nelson et al., 2001).

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