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CHAPTER 7

Thermoplasmatales

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Introduction

The order Thermoplasmatales (Reysenbach, 2001) is represented by facultatively anaerobic, thermoacidophilic, autotrophic or heterotrophic organisms that are unique among the Archaea both by their morphology and by their phylogenetic position. So far, the order harbors three families, each represented by one genus: the Thermoplasmaceae (genus *Thermoplasma*; Darland et al., 1970), the Picrophilaceae (genus *Picrophilus*; Schleper et al., 1995), and the recently described Ferroplasmaceae (genus *Ferroplasma*; Golyshina et al., 2000), formerly named *Ferromonas metallovorans*.

Thermoplasma spp. are devoid of a cell wall or envelope. For that reason, the genus *Thermoplasma*, which was for a long time (until 1995) the only member of the group, was first considered to be associated with the (bacterial) mycoplasmas (Darland et al., 1970; Masover and Hayflick, 1981). However, results of 16S rRNA sequence analyses revealed that *Thermoplasma* was a member of the archaeal domain (Woese and Fox, 1977; Woese et al., 1980; Woese et al., 1990). Although the sequences were quite unique, they clustered within the kingdom Euryarchaeota (Woese et al., 1990) and most calculations placed them between the Methanobacteriales and the Archaeoglobales. Furthermore, the affiliation to the Archaea was clear from a number of biochemical and molecular features (Stetter and Zillig, 1985; Langworthy and Smith, 1989). However, several characteristics were more crenarchaeotal than euryarchaeotal, like the physiology (Darland et al., 1970; Belly et al., 1973; Stetter and Zillig, 1985; Segerer et al., 1988) and the composition of the DNA-dependent RNA polymerase (Sturm et al., 1980; Zillig et al., 1982). In contrast, the requirement for polypeptide synthesis and the degree of stability of the ribosomal subunit association (Londei et al., 1986) supported the relationship to the Euryarchaeota, and the structure of the RNA polymerase of *Thermoplasma* was taken to be possibly of no phylogenetic significance (Yang et al., 1985). The lack of a cell wall as well as some other properties resembling those of mycoplasmas, e.g., the shape of the colonies, was therefore due to convergent evolution of these entirely unrelated groups of organisms. At present, there is no doubt that *Thermoplasma*, together with *Picrophilus* and *Ferroplasma*, represents a separate order within the domain Archaea.

Phylogeny

Based on 16S rRNA sequence data, the order Thermoplasmatales is a member of the euryarchaeotal branch of the Archaea (Woese et al., 1990). It forms an isolated cluster which branches in most calculation programs between the Methanobacteriales and the Methanomicrobiales/Halophiles (Fig. 1). However, the 16S rRNAs of all members show an unusual nucleotide sequence (for *Thermoplasma*, see Woese et al., 1980) and a high number of base exchanges in comparison to all other Archaea known so far. As a consequence the Thermoproteales exhibit low phylogenetic similarities (between 0.6 and 0.73 to all other Euryarchaeota and 0.58 to 0.67 to the Crenarchaeota). These values indicate that at the moment, no closer relatives of the members of the Thermoplasmatales are described. Within the order, the representatives of the different genera exhibit phylogenetic similarities between 0.86 and 0.89 (with the exception of the two *Thermoplasma* species, which show an identity of 98.6%).

Taxonomy

The order Thermoplasmatales (including the family Thermoplasmaceae) was first defined in the latest edition of *Bergey's Systematic Bacteriology* (Reysenbach, 2001). It is comprised of three different families, each represented by one single genus. So far, the genus *Thermoplasma* (Darland et al., 1970), the type genus for the family Thermoplasmaceae and the order Thermoplasmatales, harbors two described species: *T. acidophilum* and *T. volcanium* (Segerer et al., 1988). The type strain for *T. acidophilum* is strain 122-1B2^T (ATCC 25905^T and DSM 1728^T;

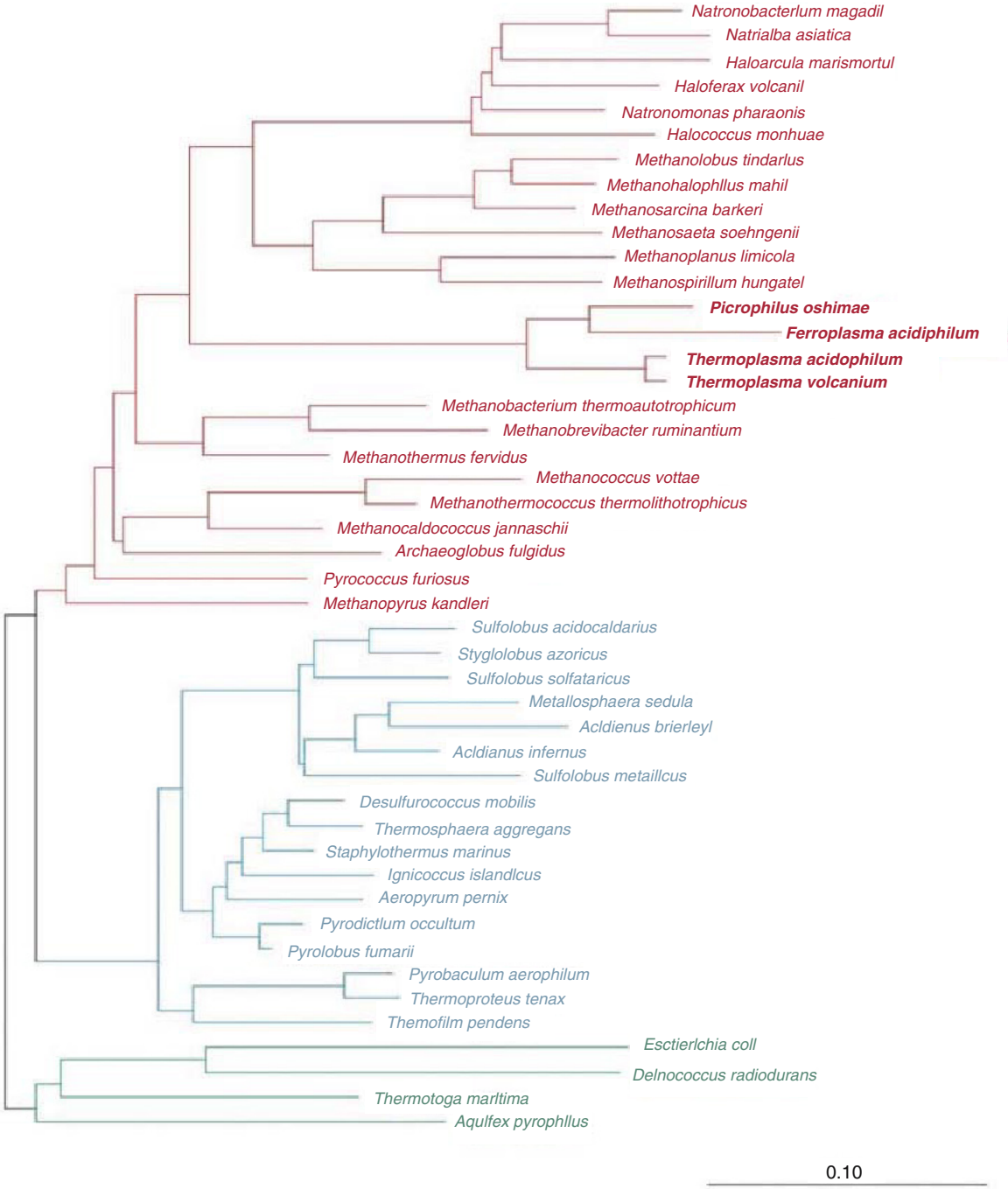

Fig. 1. Phylogenetic tree based on 16S rRNA sequences. The tree was calculated using the neighbor-joining program with Jukes-Cantor correction, which is included in the ARB package (Technische Universität, München; Ludwig and Strunk, 1997). Scale bar: 10 estimated exchanges within 100 nucleotides. Red lines = Euryarchaeota; Blue lines = Crenarchaeota; and Green lines = Bacteria.

American Type Culture Collection [ATCC], Rockville, MD, USA; Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH [DSMZ], Braunschweig, Germany) and for *T. volcanium* strain GSS1*^{T} (ATCC 51530^{T} and DSM 4299^{T}). The latter presents the DNA homology group 1 of *T. volcanium*. Additionally, two further DNA homology groups within *T. volcanium* have been established: strains KD3 (representing DNA homology group 2) and KO2 (representing DNA homology group 3), which are available from the DSMZ (DSM 4300 and 4301, respectively). They do not hybridize significantly with organisms from the other groups

(Segerer et al., 1988), indicating that the organisms are genomically unrelated (Schleifer and Stackebrandt, 1983). However, owing to the lack of distinctive phenotypic features, a description of separate species was not carried out (Segerer et al., 1988). The representatives of the three homology groups are thus still treated as the single taxon, *T. volcanium*. The two *Thermoplasma* species can be distinguished by the G+C content of their genomic DNA and by DNA-DNA hybridization (Table 1). *Thermoplasma acidophilum* exhibits a G+C content of 46 mol% (Christiansen et al., 1975; Searcy and Doyle, 1975b), whereas the G+C content of *T. volcanium* is ~38 mol%. There is no significant DNA homology between the two species.

The genus *Picrophilus* represents the second family of the Thermoplasmatales, the Picrophilaceae (Schleper et al., 1996). Two *Picrophilus* species are described: *P. oshimae* (type strain: KAW $2/2^T$, DSM 9789^T) and *P. torridus* (type strain KAW $2/3^T$, DSM9790^T). However, since in the original papers (Schleper et al., 1995; Schleper et al., 1996) only poor information is given for the second species (*P. torridus*), their differentiation is not very clear. It is stated that *P. torridus* grows "significantly faster" than *P. oshimae*, contains no plasmids (which is however also true for some *P. oshimae* strains) and has a different DNA restriction pattern, although it resembles that of *P. oshimae*. Furthermore the 16S rRNA shows 3% difference within the first 250 positions. However, the sequence of *P. torridus* is still not available in the databases.

The third family, the Ferroplasmaceae, harbors one genus, *Ferroplasma*, with two species, *F. acidiphilum* type strain Y^T (DSM 12658^T; Golyshina et al., 2000) and "*F. acidarmanus*" (Edwards et al., 2000). However, the latter is so far not validly described. The two species can be distinguished by the ability of "*F. acidarmanus*" to grow heterotrophically on yeast extract, whereas *F. acidiphilum* is unable to use yeast extract as sole energy source. Furthermore, the pH optima and range differ significantly (1.2 for "*F. acidarmanus*," range 0–2.5, and 1.7 for *F. acidiphilum*, range 1.3–2.2; Table 1). In 16S rRNA sequence analysis, no differences between both species were obtained.

Habitat

The first representative of the genus *Thermoplasma*, *T. acidophilum*, was isolated aerobically from a coal refuse pile in Indiana, in the United States (Darland et al., 1970). Further isolates were also obtained from self-heated smoldering coal refuse piles in the United States and from water samples at these locations (Belly et al., 1973; Brock, 1978). Although smouldering coal piles were obviously colonized within a short time after ignition (Belly et al., 1973; Brock, 1978) because of their anthropogenic origin, it appeared unlikely that they represent the primary habitat of the organism. A first natural habitat of *Thermoplasma* was reported in 1982, the occurrence of *Thermoplasma* in a Japanese hot spring (Ohba and Oshima, 1982). However, no further details had been published. A broad screening for *Thermoplasma* in numerous solfataric fields in Italy (Naples; Figs. 2–4; and Vulcano Island; Figs. 5 and 6), the United States (Yellowstone National Park, Wyoming), Iceland

Table 1. Differentiation of the species within the order Thermoplasmatales.

Properties	*Thermoplasma acidophilum*	*Thermoplasma volcanium*	*Picrophilus oshimae*	*Picrophilus torridus*	*Ferroplasma acidiphilum*	*"Ferroplasma acidarmanus"*
Morphology, size of cells	Pleomorphic, 0.2–5µm	Pleomorphic, 0.2–5µm	Irregular cocci, 1–1.5µm	Irregular cocci, 1–1.5µm	Pleomorphic, 1–3µm long, 0.3–1µm wide	Pleomorphic
Flagella	+	+	+	+	–	n.d.
Autotrophy	–	–	–	–	+ (needs vitamin solution)	+ and heterotrophic
Relation to oxygen	Facultatively aerobic	Facultatively aerobic	Aerobic	Aerobic	Aerobic	Aerobic
Optimal growth temperature (°C)	59	60	60	60	35	~37
Temperature range (°C)	45–63	33–67	47–65	47–65	15–45	n.d.
Optimal pH	1–2	2	0.7	0.7	1.7	1.2
pH range	0.5–4	1–4	0–3.5	0–3.5	1.3–2.2	0–2.5
G+C content of genomic DNA (mol%)	46	38–40	36	n.d.	36.5	n.d.

n.d. = no data available.

Fig. 2. "Solfatara" crater near Naples, Italy.

Fig. 3. The small, highly active solfataric area at "Pisciarelli Solfatara," Naples, Italy.

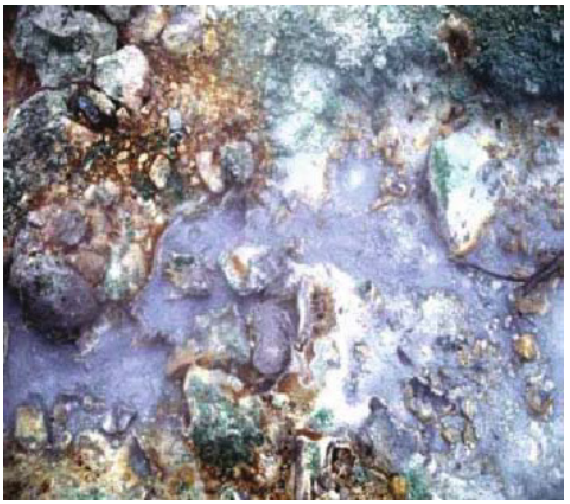

Fig. 4. Sampling site at "Pisciarelli Solfatara" with *Cyanidium caldarum*: pH 2.0; temperature 55°C.

Fig. 5. The island Vulcano, Italy, with the active solfataric area (in the center) and the Vulcanello (in the background).

(Krisuvik), the Azores (Furnas), and Indonesia (Java) demonstrated that aerobic and anaerobic zones of continental volcanic areas are the natural habitat of these organisms (Segerer et al., 1988). In these studies, an additional isolate was obtained from a warm, acidic, tropical swamp in Java (Segerer et al., 1988). Although there was evidence that *Thermoplasma* can also thrive within marine hydrothermal systems, like the submarine solfatara field close to the beach on Vulcano Island, Italy (Segerer et al., 1988), no isolates from deep-sea "black smoker" vents have been obtained so far (A. Segerer, unpublished observation).

Both *Picrophilus* strains were isolated from geothermal solfataric soils and springs in Hokkaido, northern Japan (Schleper et al., 1996). Therefore, they share in general the same biotopes as *Thermoplasma*, although their pH minima and optima are significantly lower. Since both *Picrophilus* species are inhibited by 0.2 M NaCl, they may be restricted to terrestrial geothermal environments.

Strains of *Ferroplasma* were isolated from a bioreactor, which was operated with gold-containing arsenopyrite/pyrite ore concentrate from Kazakhstan (Golyshina et al., 2000) and from a pyrite ore-body at Iron Mountain, California, United States (Edwards et al., 2000). In addition, 16S rRNA genes were detected in an acidic geothermal pool on the Caribbean island

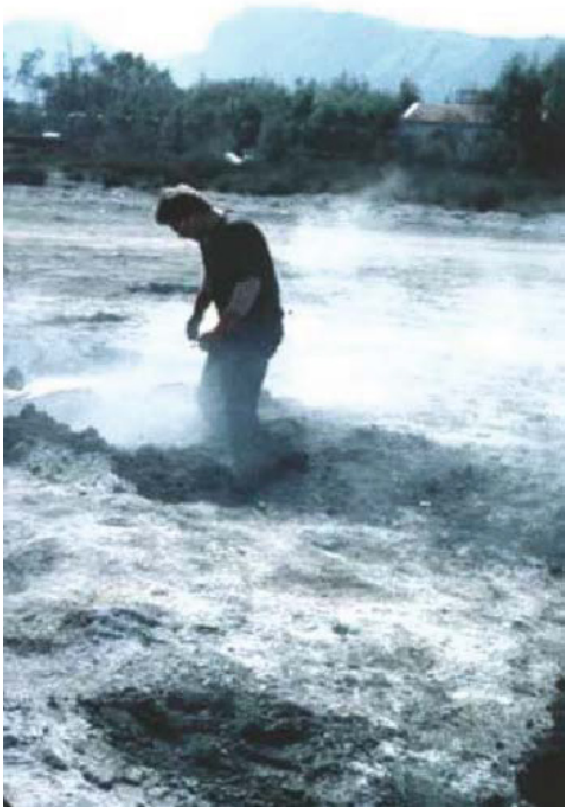

Fig. 6. Sampling at the solfataric fields at Vulcano, Italy.

of Montserrat (Burton and Norris, 2000). Since both isolated organisms grow at temperatures around 37°C and have been isolated from highly distant locations, they may occur in many sulfidic ore-containing mines and heaps on earth.

Isolation

Enrichment

Several procedures to obtain *Thermoplasma* strains have been described. They can be enriched by aerobic incubation of samples in Darland's culture medium (see recipe below). Although low pH and high temperature are highly selective, overgrowth of rod-shaped, sporeforming, bacterial contaminants has been observed in many cases (Belly et al., 1973; Segerer et al., 1988), and good results were only obtained by adding appropriate antibiotics (e.g., vancomycin) to the cultures. Alternatively, the medium must be adjusted to pH 1 to prevent bacterial growth (Belly et al., 1973).

Alternatively, water samples were passed through a 0.45 μm filter followed by a passage through a 0.22 μm filter. Subsequent incubation of the latter in culture medium yielded pure cultures of *Thermoplasma* (Belly et al., 1973).

A highly selective and convenient procedure for the enrichment of *Thermoplasma* is incubation of samples in anaerobic media (Balch et al., 1979) in the presence of elemental sulfur (Segerer et al., 1988). Darland's medium, pH 2, was supplemented with 0.4% (w/v) of sulfur and incubated with an aliquot of the sample in rubber-stoppered serum bottles containing either a N_2 or N_2/CO_2 (80:20, v/v) atmosphere. The gas phase should be devoid of H_2 to prevent growth of facultatively or strictly anaerobic representatives of the order Sulfolobales, e.g., *Acidianus* or *Stygiolobus* strains. In enrichment cultures obtained by using this procedure, no contaminants were detected by light microscopy or plating (Searcy and Doyle, 1975a).

For the enrichment of *Picrophilus*, an aerobic growth medium described by Smith et al. (1975) was used (see below). Yeast extract (0.1%, w/v) and glucose (1%, w/v) served as carbon sources. Incubation was carried out at pH 1 at 60°C.

Strains of *Ferroplasma* were enriched in a modified 9K-medium (see below; Silverman and Lundgren, 1959; Golyshina et al., 2000) at pH 1.6–1.9 and at incubation temperatures between 28 and 30°C. Alternatively, enrichment in a medium according to Edwards et al. (1998) with a pH of 1 and at 37°C was described, using pyrite as energy source (Edwards et al., 2000). Since yeast extract is essential for the growth of both strains, this (0.02%, w/v) must be added to the culture media.

Isolation Procedures

For the isolation of *Thermoplasma*, Darland's culture medium (see below) is inoculated with samples (5% inoculum) and incubated at 59°C. *Thermoplasma* growth occurs within 2 days to 3 weeks. Isolation is achieved by subsequent plating, which is conveniently performed under aerobic conditions. Therefore, it is recommended that enriched cultures obtained by using the anaerobic enrichment procedure (see above) should be transferred once into aerobic liquid medium before plating. Alternatively, pure cultures can be obtained by thrice-repeated serial dilutions. However, the fastest and most secure isolation procedure is the separation by the use of optical tweezers (Huber et al., 1995).

Picrophilus strains were isolated by plating on 12.5% starch plates (pH 1) containing yeast extract and glucose. Colonies were obtained after 6 days at an incubation temperature of 60°C. Both strains of *Picrophilus* were isolated by serial dilutions. So far, nothing is published about growth on solidified media.

Ferroplasma has been isolated from a bioreactor and from an enrichment culture out of a pyrite ore-body by the use of serial dilutions. Growth on solidified media is so far not documented.

Identification

All representatives of the order are extremely acidophilic, growing optimally at pH < 2. The lack of a cell wall is characteristic for the members of the genera *Thermoplasma* and *Ferroplasma*, while *Picrophilus* possesses an outer S-layer. *Thermoplasma* and *Picrophilus* are further characterized by their thermophily, exhibiting temperature optima around 60°C. All organisms stain Gram negative and lack resting stages.

The diameter of *Thermoplasma* and *Ferroplasma* cells, ranging from 0.2 to roughly 5 μm (Table 1), is highly variable. The same is true for the cell shape: filamentous, disc, and club (Fig. 7), and coccoid forms occur concomitantly in the same culture, making it difficult to ensure purity of the strain by microscopic investigations. Filamentous cells are especially abundant in the early exponential growth phase, while coccoid forms predominate in the stationary phase. Buds, about 0.3 μm in width, are often associated with mother cells. In *Ferroplasma*, the buds appear tubular or vesicular in shape, tending to form septation annuli. The tubular extrusions range from 85 to 142 nm in diameter and up to 1 μm in length (Golyshina et al., 2000). *Thermoplasma* and *Ferroplasma* cells are readily discernible from all other thermoacidophiles by the unique feature of lacking a true cell wall (Fig. 8). They are surrounded by a single triple-layer membrane about 4–10 nm thick. *Thermoplasma* shows a pale yellowish-green fluorescence under ultraviolet (UV) radiation in the fluorescence microscope (Segerer et al., 1988). Despite the lack of a cell wall, the cells are flagellated and motile (Black et al., 1979; Segerer et al., 1988). Usually, monopolar, monotrichous flagellation is found, but sometimes multiflagellated cells also were observed (Segerer et al., 1988). On solid media, colonies of *Thermoplasma* are usually small (about 0.5 mm in diameter) and are either colorless or brownish. As in the *Mycoplasmas*, fully grown colonies resemble a fried egg in shape (Darland et al., 1970; Belly et al., 1973).

Cells of *Picrophilus* are irregular cocci, 1 to 1.5 μm in diameter (Table 1). In exponentially growing cultures, many cells are present in incompletely divided division forms of two or three individuals. Zones of low electron density were found after thin sectioning, resembling vacuoles, which were however not separated from the cytoplasm by a membrane. A 40-nm thick S-layer, which is situated on top of the cytoplasmic membrane, distinguishes *Picrophilus* from the other members of the Thermoplasmatales. The S-layer has a tetragonal symmetry (center-to-center distance about 20 nm) and consists of an outer dense and inner, almost empty stratum consisting of widely spaced pillars that anchor the surface layer in the membrane. No flagella or pili have been observed (Schleper et al., 1995).

All members of the Thermoplasmatales are resistant to cell wall inhibitors (like ampicillin or vancomycin) and to streptomycin. While *Picrophilus* is sensitive to chloramphenicol and

Fig. 7. Electron micrograph of cells of *Thermoplasma* acidophilum DSM 1728^T, Pt-shadowed. Bar 1 μm.

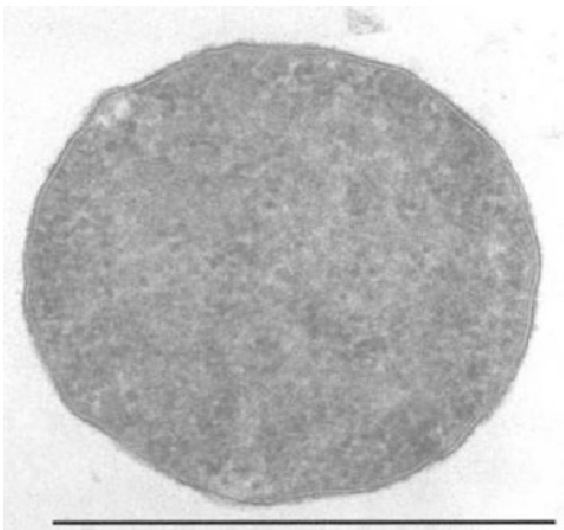

Fig. 8. Electron micrograph of a thin section of *Thermoplasma* acidophilum. Bar 1 μm. (The photograph was kindly provided by H. Engelhardt.)

novobiocin, the other organisms are resistant to these antibiotics.

Many molecular investigations were carried out with *Thermoplasma*, especially with *T. acidophilum*. It turned out that it contains a number of unique and characteristic chemical compounds. An unusual mannose-rich, 152-kDa glycoprotein is located in the cell membrane and is thought to form a hydroskeleton via a network of sugar residues surrounding the cell, thus contributing to the remarkable rigidity of the membrane (Yang and Haug, 1979). The membrane lipids contain predominantly acyclic, mono- and bi- pentacyclic C_{40} biphytanyl diglycerol tetraethers along with small amounts of C_{20} phytanyl glycerol diethers (Langworthy, 1977; Langworthy, 1985; Langworthy et al., 1982; Langworthy and Pond, 1986). The structure of a polar tetraether lipid was fully established and shown to be a lipoglycan (MW 5,300), consisting of 24 mannosyl residues and one glucosyl residue bound to a diglyceryltetraether (Mayberry-Carson et al., 1974; Smith, 1980). Several novel neutral glycolipids, consisting of caldarchaeol (dibiphytanyl-diglycerol tetraethers) and monosaccharide residues (gulose and glucose) on one side or both sides of the core lipids, were described recently for *T. acidophilum* (Uda et al., 1999). *Picrophilus* has similar lipids, with the exception that a β-glycosyl residue is present in the major lipid component. In contrast, *Ferroplasma* contains no tetraethers. The main phospholipid is archaetidyl glycerol. Furthermore, archaetidic acid and dimers of both ether lipids were found in small amounts (Schleper et al., 1995).

The DNA-dependent RNA polymerase of *Thermoplasma* consists of seven subunits and is resistant to rifampicin, streptolydigin and α-amanitin (Sturm et al., 1980). In the corresponding enzyme of *Picrophilus*, several components were absent (e.g., A″ and E) and the enzyme does not crossreact in immunodiffusion assays with antibodies against the *Thermoplasma* enzyme (Schleper et al., 1995). The respiratory chain of *Thermoplasma* contains at least one *b*-type cytochrome (Belly et al., 1973; Holländer, 1978; Searcy and Whatley, 1982) and several quinones (Holländer et al., 1977; Collins and Langworthy, 1983). Among the quinones, a characteristic compound called "thermoplasmaquinone" was found, which was not detected in other Archaea. In addition, menaquinone and methionaquinone are present (Shimada et al., 2001). Coenzyme F_{420} is present in about 1% of the amount typical for methanogens (Lin and White, 1986). The DNA is associated with a small basic histone called "Hta" that exhibits partial amino acid sequence homology to the HU-1 protein of *Escherichia coli* and to the calf thymus histones H3 and H2A (Searcy, 1975a; Searcy and DeLange, 1980; DeLange et al., 1981. *Thermoplasma acidophilum* possesses a protein that resembles the human ubiquitin (Wolf et al., 1993). Furthermore, it contains proteasomes, which function as threonine proteases in ATP-dependent proteolysis (Seemüller et al., 1995). Structural investigations on the thermosome, which represents the archaeal chaperonin, were carried out by electron cryo-microscopy and by high resolution analysis (2.6 Å) of protein crystals (Nitsch et al., 1997; Ditzel et al., 1998). Based on these results, the molecule is composed of two stacked eight-membered rings of alternating α- and β-subunits. Studies on the mechanism of its ATPase activity revealed that the thermosome had unique allosteric properties (Gutsche et al., 2000).

For *Picrophilus*, bioenergetic studies with liposomes indicate an intrinsic instability of the cytoplasma membrane at higher pH values, resulting in a loss of viability and cell integrity above pH 4 (van de Vossenberg et al., 1998).

Cultivation

Medium for *Thermaplasma* Species

Darland's medium is suitable for the growth of all strains of *Thermoplasma*.

Darland's Medium (Darland et al., 1970)

KH_2PO_4	3.00 g
$MgSO_4 \cdot 7H_2O$	1.02 g
$CaCl_2 \cdot 2H_2O$	0.25 g
$(NH_4)_2SO_4$	0.20 g
Yeast extract	1.00 g
Glucose · H_2O	10.0 g

The mineral base of the medium is dissolved in one liter of double-distilled water and the pH is adjusted to around 2 with 10% (v/v) H_2SO_4.

For aerobic cultivation, 30-ml aliquots are distributed into 100-ml Erlenmeyer flasks equipped with an air cooler (e.g., a 1-ml pipette tightly fitted through a rubber stopper) and autoclaved. Yeast extract, glucose, and (if necessary) meat extract are added separately from sterile stock solutions. Reduction of the glucose content to 0.5% (w/v) does not significantly affect growth and cell yield. For reasons of convenience, we prepare a stock mixture of 30% (w/v) glucose and 6% (w/v) yeast extract (plus, if desired, 3% [w/v] meat extract), which is sterilized by passage through a 0.2-µm filter membrane. From this stock, 0.5-ml are added to each 30 ml of mineral base medium.

For anaerobic cultivation of *Thermoplasma* strains, the medium must be supplemented with about 0.4% (w/v) elemental sulfur. It is distributed as 15-ml aliquots into 100-ml serum bottles. The atmosphere (~150 kPa) may consist of N_2, N_2/CO_2 (80:20 v/v), or H_2/CO_2 (80:20 v/v). Serum bottles containing elemental sulfur must be sterilized by Tyndall's fractional sterilization procedure.

Aerobic and anaerobic media for the strains of *T. volcanium* group 3 (see "Identification") should be supplemented with 0.025–0.05% (w/v) meat extract in addition to yeast extract and glucose.

Medium for *Picrophilus* Species

This medium was originally described by Smith et al. (1975) according to E. A. Freundt for the cultivation of *Thermoplasma acidophilum* (Schleper et al., 1995). It is in principle similar to Darland's medium, with the exception of the absence of KH_2PO_4.

$(NH_4)_2SO_4$	0.20 g
$MgSO_4 \cdot 7H_2O$	0.50 g
$CaCl_2 \cdot 2H_2O$	0.25 g
Yeast extract	1.00 g

The pH is adjusted to around 2 with 10 N H_2SO_4. After autoclaving, glucose is added to a final concentration of 1%.

MEDIUM FOR *FERROPLASMA* SPECIES A modified 9K medium (Silverman and Lundgren, 1959) is used for the cultivation of *Ferroplasma* (Golyshina et al., 2000).

$MgSO_4 \cdot 7H_2O$	0.40 g
$(NH_4)_2SO_4$	0.20 g
KCl	0.10 g
K_2HPO_4	0.10 g
$FeSO_4 \cdot 7H_2O$	25.0 g
Yeast extract	0.20 g
Trace elements	1.0 ml

The pH is adjusted to 1.7 with 10% (v/v) H_2SO_4. Trace elements are described by Segerer and Stetter (1992b). For the growth of both *Ferroplasma* strains, ferrous sulfide can be replaced by pyrite.

Cultivation on Plates

For growth on solidified media, best results are obtained for members of the Thermoplasmatales using 10–12% starch (e.g., Stärke Gel, Serva). Only poor results were obtained by plating *Thermoplasma* spp. onto agar or Gelrite plates (Searcy and Doyle, 1975a). It is recommended to adjust the pH to about 3 just before pouring the plates. High and reproducible yields (plating efficiency ~90 to 100%) were obtained by incubating the plates in a pressure cylinder (Balch et al., 1979) microaerobically, in a humid atmosphere consisting of roughly 60% air and 40% CO_2 (v/v). Small, colorless to brownish colonies showing the "fried egg" appearance typical of *Thermoplasma* emerged within 7 or more days.

Cultivation Conditions

Strains of *Thermoplasma* (*Picrophilus* and *Ferroplasma*) can be cultivated in a wide variety of glass bottles and fermentors. Special attention has to be paid to the construction of fermentors because of the high corrosivity of the medium due to the low pH, elevated temperature, and (in case of anaerobic cultivation) high amounts of H_2S produced during growth. Therefore, only fermentors containing high-quality steel are recommended.

All *Thermoplasma* strains investigated thus far are best grown at 57–59°C and at around pH 2, whereas for *Picrophilus*, the optimal pH is 0.7. Optimal growth for *Ferroplasma* strains occurs between pH 1.2 ("*F. acidarmanus*") and 1.7 (*F. acidiphilum*), with temperature optima of 40 and 35°C, respectively.

Preservation

Cultures of *Thermoplasma* are significantly more resistant to storage procedures when grown anaerobically. The reason for this phenomenon, which is also observed with *Acidianus* spp. (Segerer et al., 1986a), is unknown. Adjustment to pH ~5 with sterile $CaCO_3$ prior to storage has a further positive influence on long-term viability, presumably because *Thermoplasma* actively maintains an internal pH of about 5 (Searcy, 1976). Possible damage to cells during storage caused by the penetration of acids is thus avoided. Therefore, for storage, the use of anaerobically grown cultures at pH ~5 and 4°C is recommended. Although viability of such cultures is sufficient even after 12 months of storage, maintenance transfer every 2–3 months is advised. Best results for long-term storage are achieved in culture media containing 5% DMSO (dimethyl sulfoxide) and storage in the gas phase over liquid nitrogen (around –140°C; H. Huber and K. O. Stetter, unpublished observation).

Viable stock cultures for *Picrophilus* can be obtained by suspending cells in basal salt medium (pH 4.5) containing 20% glycerol. These suspensions can be kept at –70°C. Neutralization of the culture medium for short-term storage is not recommended because *Picrophilus* lyses above pH 5 (Schleper et al., 1995).

Nothing is stated in the original papers on preservation results for *Ferroplasma* (Golyshina et al., 2000; Edwards et al., 2000). However, it should be possible to store these strains in fresh medium supplemented with 5% DMSO over liquid nitrogen.

Physiology

The members of the genus *Thermoplasma* are obligately heterotrophic, facultatively anaerobic thermoacidophiles that require the presence of yeast extract or similar extracts for growth. At growth-limiting concentrations of yeast extract, the carbohydrates sucrose, glucose, mannose, galactose, and fructose were found to stimulate growth significantly (Belly et al., 1973; Brock, 1978). However, no growth occurs on sugars

alone or on peptone, tryptone, casamino acids, various amino acids, and alcohols. As shown by Smith et al. (1975), the growth factor(s) present in yeast extract is most likely a basic oligopeptide consisting of 8–10 amino acids. Although *Thermoplasma* has an absolute requirement for yeast extract (Langworthy and Smith, 1989), this organism also grows in the presence of meat extract or bacterial extracts (Segerer et al., 1988), suggesting that the same or similar growth factors are present in those extracts. Therefore, the nutrition of *Thermoplasma* in its natural habitat is most likely based on the products of decomposing cells of organisms sharing the biotope, e.g., *Acidianus brierleyi* (Brierley and Brierley, 1973; Segerer et al., 1986a), *Bacillus acidocaldarius* (Darland and Brock, 1971), *Cyanidium caldarium* (Geitler and Ruttner, 1936), or *Dactylaria gallopava* (Tansey and Brock, 1973). Additionally, *Thermoplasma* was also found to grow poorly on an extract of coal refuse material (Bohlool and Brock, 1974). However, it is not clear from this study whether the coal itself provides all necessary nutrients or not.

Glucose degradation was investigated in T. acidophilum. Although contradictory results were obtained, degradation appears to take place via a modified Entner-Doudoroff pathway involving nonphosphorylated intermediates, rather than via the pentose phosphate pathway or glycolysis (Searcy and Whatley, 1984; Budgen and Danson, 1986). By using D-[U-^{14}C]-glucose as tracer, CO_2 and acetic acid were detected as metabolic products, in addition to the respiration of cell extracts by key intermediates of the citric acid cycle and the presence of the enzymes malate dehydrogenase and citrate synthase. This suggests the operation of a Krebs cycle (Searcy and Whatley, 1984; Grossebüter and Görisch, 1985; Grossebüter et al., 1986).

Thermoplasma spp. grow as facultative anaerobes on molecular sulfur by sulfur respiration, forming large amounts of H_2S (Segerer et al., 1986b; Segerer et al., 1988). This feature was in the beginning unknown, and the organism was considered to be a strict aerobe (Darland et al., 1970; Langworthy and Smith, 1989). Low cell densities are obtained when the cells are grown anaerobically without sulfur, indicating the presence of further unknown electron acceptor(s). Sulfur is not a prerequisite for aerobic cultivation. When *Thermoplasma* was grown aerobically in the presence of sulfur, no formation of H_2SO_4 was found (Brock, 1978; Segerer et al., 1988), a feature typical for members of the genus *Acidianus* (Segerer et al., 1986a). In addition, no growth occurs on ferrous iron.

The growth temperatures range from about 45–67°C for *T. acidophilum* and from about 33–67°C for *T. volcanium*. The optimum growth temperature is around 59°C. Both species grow within a pH range of 0.5–4, with an optimum around pH 2 (Table 1), but growth is very slow at both extremes.

Cells lyse at neutral pH, indicating that *Thermoplasma* has an absolute requirement for protons. They cannot be replaced by other monovalent or divalent ions. Nevertheless, the internal pH is near neutrality (Hsung and Haug, 1975; Searcy, 1976). The cells do not lyse in distilled water or during heating up to 100°C. However, cells are rapidly disintegrated in the presence of sodium dodecyl sulfate (SDS).

The representatives of *Picrophilus* are obligately aerobic heterotrophs. They cannot grow by fermentation or by chemolithotrophic pathways, like sulfur respiration. Yeast extract (0.1–0.5%) serves as an energy source, yielding cell densities up to 5×10^8 cells/ml. A slight stimulation is achieved by addition of 1% glucose, sucrose or lactose. No growth occurs on these sugars alone, on starch, or on casamino acids. Growth is inhibited by the addition of relatively small amounts of NaCl (0.2 M). *Picrophilus* strains are thermophilic, exhibiting temperature optima around 60°C. No growth occurs at 40°C and below and at 67°C or above. The optimal pH is 0.7, and no growth is obtained at pH 3.5 or above, indicating that the organisms are "hyperacidophilic." Even at pH 0, cell division occurs (Schleper et al., 1995).

In contrast to the other genera, *Ferroplasma* harbors also chemolithoautotrophic organisms. *Ferroplasma acidophilum* is able to use CO_2 as a carbon source and ferrous iron or pyrite as an energy source. Ferric ion (Fe^{3+}) is the end product of the oxidation. In addition, Mn^{2+} can be oxidized. No growth occurs on other sulfidic ores or reduced sulfur compounds like elemental sulfur, thiosulfate or tetrathionate. No growth could be observed on organic substrates, although the addition of yeast extract is essential for cell propagation (Golyshina et al., 2000). It was detected that yeast extract can be replaced by a vitamin solution. "*Ferroplasma acidarmanus*" is able to grow in addition heterotrophically on yeast extract as sole energy source. It grows between pH 0 and 2.5, with an optimum at 1.2 (*F. acidiphilum* pH 1.7–2.2, optimum 1.7; Edwards et al., 2000). Both organisms are mesophiles with a temperature optimum around 37°C (Table 1).

Genetics

The whole genomes of *Thermoplasma acidophilum* and *Thermoplasma volcanium* were sequenced recently (Ruepp et al., 2000; Kawashima et al., 2000). The genomes have sizes of only 1,565 kb and 1,585 kb, respectively, being sone of

the smallest among free-living organisms. In *T. acidophilum*, 1,509 ORFs were identified, and about 16% have so far no database match. Each of the three ribosomal RNA genes is present in one copy, but the three genes are dispersed in the genome. Analyses of the data revealed that *T. acidophilum* is a typical member of the Euryarchaeota, although the highest number of ORFs (17%) was most similar to proteins of *Sulfolobus solfataricus*. The complete sequence also showed that *Thermoplasma acidophilum* is a typical Archaeon and therefore most likely not a direct ancestor of the eukaryotic cytoplasm (Ruepp et al., 2000). In general, it turned out that two classes of genes can be distinguished: the "housekeeping" genes reflect generally the phylogenetic origin, while the "lifestyle" genes (mostly genes related to metabolism) are influenced by the specific environment (Ruepp et al., 2000). In *T. volcanium*, 1,524 genes were identified. The main goal of this genome sequencing was the investigation of a correlation between higher growth temperature and genomic organization (Kawashima et al., 2000).

Ecology

Representatives of the Thermoplasmatales thrive in highly acidic biotopes, covering temperatures from 20 up to 60°C. While *Thermoplasma* and *Picrophilus* are typical inhabitants of heated solfataric areas, recent results document that *Ferroplasma* seems to be widely distributed in pyrite-dominated ore-containing habitats at temperatures around 37°C (Bond et al., 2000; Vásquez et al., 1999). So far, these biotopes were thought to be dominated by bacteria, like *Thiobacillus ferrooxidans* (now *Acidithiobacillus ferrooxidans*; Kelly and Wood, 2000), *Leptospirillum ferrooxidans*, or *Acidiphilum*. However, especially for lower pH regions in mines, ore bodies, or drainage waters, *Ferroplasma* seems to be more important than the other organisms.

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