

28 The Family *Thermoplasmataceae*

Anna-Louise Reysenbach · Kristen Brileya

Department of Biology, Portland State University, Portland, OR, USA

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Abstract

The genus *Thermoplasma* is the only described genus of the family *Thermoplasmataceae* and is represented by two species, *T. volcanium* and *T. acidophilum*. These facultative aerobes morphologically resemble bacterial mycoplasmas in that they do not have a cell wall or S-layer and form colonies that have a “fried-egg” appearance on agar. The *Thermoplasma* are obligate thermoacidophilic heterotrophs, growing optimally at 60 °C and pH 2, and are motile pleomorphic cocci. Both species genomes have been sequenced, providing additional insight to their thermoacidophilic lifestyle. Although *T. acidophilum* is a Euryarchaeota, it shares 58 % gene homology to the crenarchaeote *Sulfolobus solfataricus*, likely a result of adaptations and lateral gene transfer events due to their shared ecological niche. 16S rRNA gene sequences related to *Thermoplasmataceae* have been found in terrestrial solfataras, deep-sea hydrothermal vents, and several other environments, suggesting a broad niche range for this family and its relatives in the order *Thermoplasmatales*.

Taxonomy, Historical, and Current

The family *Thermoplasmataceae* is one of the three described families within the *Thermoplasmatales* and is represented by a single genus, *Thermoplasma*, and two species, *T. volcanium* strain GSS1 (ATCC 51530, DSMZ 4299) and *T. acidophilum* strain 122-1B2 (ATCC 25905, DSMZ 1728) (Reysenbach 2001). There is a related monospecific clade represented by *Thermogymnomonas acidicola*, however based on its 16S rRNA sequence, is probably not a *Thermoplasmataceae*, and most likely represents another undescribed family within the *Thermoplasmatales*. The members of *Thermoplasmataceae* can be distinguished from *Picrophilaceae* in that they have no S-layer and are devoid of a cell wall. Because of this characteristic, before the advent of 16S rRNA sequencing, *Thermoplasma* was thought to belong to the bacterial mycoplasmas.

They can be distinguished from members of the *Ferroplasmaceae* in 16S rRNA sequence but also in their optimum temperature for growth. The cells are highly pleomorphic, are facultative aerobes, and grow best around 60 °C and at a pH of about 2. Although the first isolates were obtained from coal refuse piles, they have been detected in terrestrial solfataras worldwide (● Fig. 28.1).

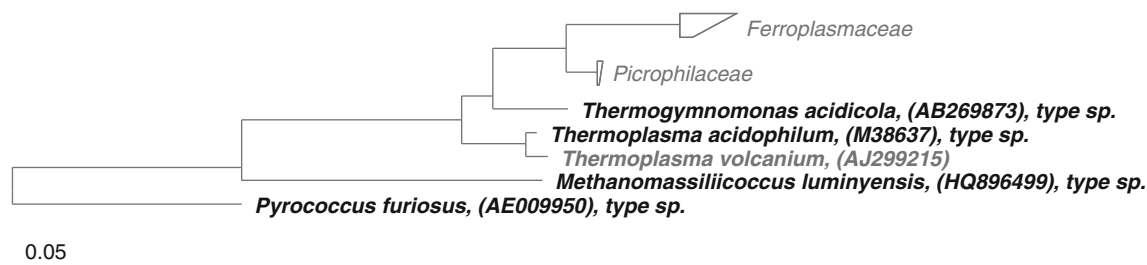
Molecular Analyses

The genomes of both *T. acidophilum* and *T. volcanium* have been sequenced and are 1.565 and 1.585 kb in size, respectively (Kawashima et al. 2000; Ruepp et al. 2000). Their coding density is 88.8 % and 86.9 %, respectively. *T. acidophilum* shares about 58 % of homologous genes with *S. solfataricus*, which suggests that even though these two organisms belong to different kingdoms in the Archaea, genes have been shared because of their similar ecological niche. About 1,509 ORFs were identified, and no plasmids were detected, although a 15.2 kbp plasmid had been reported in other isolates of *T. acidophilum* (Yasuda et al. 1995).

Like *Picrophilus*, *T. acidophilum* does not have any homologs for the essential nucleotide excision repair (NER) nuclease, UvrABC proteins, nor homologs for reverse gyrase and histone-like proteins. How these organisms repair their DNA or keep it intact in these extreme low pH and high temperature conditions is unclear, although Moen et al. (2011) showed that *T. acidophilum* has a uracil base excision repair (BER) pathway. *T. acidophilum* can do short-patch repairs as seen in *Archaeoglobus fulgidus* but also can repair about 15 nucleotides using an ATP-/ADP-stimulated long-patch BER. Furthermore, DNA stability may be conferred by histone analogs closely related to bacterial proteins that bind to DNA (Ruepp et al. 2000).

Glucose assimilation occurs by a non-phosphorylated variant of the Entner–Doudoroff pathway, and the acetyl-CoA enters through the oxidative tricarboxylic acid cycle (TCA). Because of the lack of homologs for enzymes such as fructose bisphosphate aldolase, the presence of a complete EMP pathway has not been confirmed (Ruepp et al. 2000).

T. acidophilum can gain energy anaerobically from sulfur respiration, but the only analogs that were found were similar to those seen in the bacterium *Salmonella typhimurium*. The *Thermoplasma* genome sequence confirmed the pathway for polypeptide degradation, with the identity of the proteasome confirmed and over 23 putative proteases identified.



■ Fig. 28.1

Phylogenetic reconstruction of the family *Thermoplasmataceae* was based on 16S rRNA and created using the maximum likelihood algorithm RAxML (Stamatakis 2006). The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; <http://www.arb-silva.de/projects/living-tree>). Representative sequences from closely related taxa were used as out-groups. Scale bar indicates estimated sequence divergence

Phenotypic Analyses

The isolates of *Thermoplasma* are all obligate acidophilic heterotrophs and facultative aerobes, growing well on yeast extract and carbohydrates such as sucrose, glucose, and mannose that stimulate growth. *Thermoplasma* species can grow anaerobically by sulfur respiration and produce H₂S in the process (Segerer et al. 1986, 1988). Both *T. acidophilum* and *T. volcanium* are highly pleomorphic cocci ranging from 0.2 to 5 μm in diameter and may appear filamentous, club, or disc shaped. The cells are motile usually by a monotrichous flagellum, but multiflagellate cells also have been observed (Segerer et al. 1988). They have optimal growth at about 60 °C, and pH 2. *T. volcanium* (33–67 °C) has a much broader temp growth range than *T. acidophilum* (45–63 °C) and differs in their respective G+C mol% content, 40 and 46 mol%, respectively. Like *Ferroplasma* and *Thermogymnomonas*, *Thermoplasma* spp. do not have a cell wall or cell envelope, but have a 7 nm thick cell membrane. Typical of all Archaea, their cell membrane lipids are composed of isoprenoid (phytyl-based) ether lipids, primarily C₄₀ biphytyl diglycerol tetraethers with small amounts of C₂₀ phytyl glycerol diether (Langworthy 1977, 1985; Langworthy and Pond 1986; Langworthy and Smith 1989). The two species' DNA does not cross-hybridize.

Isolation, Enrichment, and Maintenance Procedures

Thermoplasma can be isolated by inoculating samples from acid solfatar soils and streams and from hot coal refuse piles into the Darland medium (Darland et al. 1970). If grown aerobically, then vancomycin (1,254 mg/ml) should be added to prevent growth of bacteria. Enrichments under anaerobic conditions are generally more successful, but require the presence of sulfur (0.4 % w/v) under an atmosphere of N₂ or N₂/CO₂. Growth is seen between a few days to several weeks at about 60 °C. Isolations can be done on agar plates under microaerophilic (60 % air, 40 % CO₂) conditions in an anaerobic chamber. Colonies will appear colorless to brown, with a “fried-egg” appearance.

New strains can be maintained better if grown anaerobically, and adjusting the pH of the medium to 5 using CaCO₃ prior to storage helps long-term storage at 4 °C. Cultures can also be stored with 5 % DMSO under a gas phase and in liquid nitrogen (Huber and Stetter 2006). If maintained aerobically, they need to be transferred routinely and kept at room temperature. Growth is best if transfers are with 10–20 % inoculum.

Ecology

Although the first *Thermoplasma* isolate, *T. acidophilum*, was isolated from hot burning coal refuse piles in Indiana, USA, they have also been detected and isolated from terrestrial solfataras as in Vulcano, Italy, Yellowstone National Park, Azores, and Indonesia. No marine *Thermoplasma* have been isolated, although a related clade in the *Thermoplasmatales* has been detected and isolated from deep-sea vents (Flores et al. 2012; Reysenbach et al. 2006). Additional *Thermoplasmatales* have been reported from 16S rRNA gene surveys in deep sediments, shallow marine surface water, acid mine drainage areas, ruminants, subgingival plaque, and acid rivers like the Rio Tinto (Horz et al. 2012; Poulsen et al. 2013; Sanchez-Andrea et al. 2011; Volant et al. 2012; B. Wemheuer et al. 2012). It is likely these are new families within the *Thermoplasmatales* or relatives of *Ferroplasma*.

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