# 24 The Family *Picrophilaceae*

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**Abstract**
The genus *Picrophilus* is the only described genus of the family *Picrophilaceae* and is represented by two species, *P. torridus* and *P. oshimae*. The only apparent differentiating feature between the two species is their 16S rRNA gene sequences which are 99.45 % similar over 1,460 nucleotides. Given this, it is unlikely that these are two separate species. *Picrophilus* are nonmotile, irregular cocci and are thermophilic, heterotrophic, obligate aerobes that are hyperacidophilic, growing optimally at 60 °C and pH 0.7. *Picrophilaceae* differs from *Ferroplasmaceae* and *Thermoplasmaceae* in that members of *Picrophilaceae* have an S-layer. The genome of *P. torridus* has been sequenced, revealing one of the smallest known genomes of an aerobic free-living heterotrophic archaeon at 1.55 Mb.

## Taxonomy, Historical, and Current

The *Picrophilaceae* are an extremely thermoacidophilic family within the order *Thermoplasmatales* and are represented by a single genus, *Picrophilus*, and two species, *P. torridus* (type strain KAW 2/3$^T$, DSM9790$^T$) and *P. oshimae* (type strain: KAW 2/2$^T$, DSM 9789$^T$). The two species have very similar 16S rRNA gene sequences (99.45 % similarity), which would suggest they are strains of the same species (Chun et al. 2007). However, the genus *Picrophilus* is 9.3–11.5 % different in 16S rRNA gene sequence to *Thermoplasma acidophilum* and forms a distinct clade within the Thermoplasmatales (Schleper et al. 1995a, 1996). The species were first isolated in 1996 from hot acidic dry soils or solfataras in Northern Japan, and no further isolations have been obtained (Schleper et al. 1995a, b, 1996). These microbes are the most acidophilic organisms currently known, with the ability to grow at a pH of 0.06. They are obligate aerobic acidophiles and are unable to maintain their membrane integrity at pH values higher than 4 (van de Vossenberg et al. 1998). Unlike their relatives, *Thermoplasma* and *Ferroplasma*, *Picrophilus* contains an S-layer cell envelope and maintains a very low cytoplasmic pH of about 4.6 (Fig. 24.1).

## Molecular Analyses

The acidic lifestyle, and unusually low internal pH of *P. torridus* (pH 4.6), provides some special challenges for this archaeum (Angelov and Liebl 2006). With the genome sequence of *P. torridus*, new insights into the extreme *thermoacidophilic* lifestyle of this organism were revealed (Genbank accession number AE017261). At only 1.55 Mb, its genome is one of the smallest known for an aerobic nonparasitic/symbiotic heterotroph determined thus far. Additionally, a 7.6 Kbp plasmid was characterized that may encode for a restriction/modification system in addition to its replication functions (Angelov et al. 2011). The genome has a very high coding density (at 91.7 %). About 74 % of the ORFs could be assigned a function, and of the 397 hypothetical ORFs, 79 were unique to *P. torridus* (Futterer et al. 2004); for reviews see Angelov and Liebl (2006) and Ciaramella et al. (2005). Interestingly, 174 of the hypothetical ORFs had orthologs to only genomes from thermoacidophiles, suggesting that most thermoacidophiles share similar genetic strategies for thriving in their hot and acidic environments.

The genome of *P. torridus* contains a large number of genes for repair and recombination, but no reverse gyrase was detected. Since *P. torridus* is an obligate aerobe, its genome also encodes for key enzymes, such as superoxide dismutase, that are important in dealing with oxygen stress. Additionally, *P. torridus* seems to be able to synthesize porphyrins like cytochromes and adenosylcobalamin (Futterer et al. 2004).

Because the organism is primarily a scavenger of peptides and proteins, it has genes encoding for extracellular proteases and several ATP-binding cassette (ABC) transporters for peptide uptake. Twelve percent of all the genes in *P. torridus* play a role in transport. Like *Thermoplasma acidophilum*, the ratio of secondary to primary transporters in *P. torridus* is unusually high compared to other organisms like *Pyrococcus horikoshii*. The secondary transporters in *P. torridus* all use protons and not $Na^+$ as the motive force for driving metabolite transport. Using the transmembrane proton gradient for transport probably assists in maintaining the huge pH difference across the cell membrane. Furthermore, the $K^+$ transporting ATPase most likely assists $K^+$ uptake and would counteract the proton influx, thereby inverting the transmembrane electrical potential to positive inside the cell. The unusually low cytoplasmic pH

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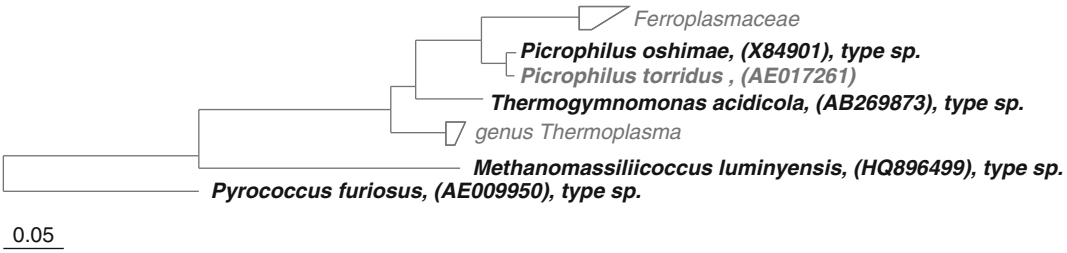


**Fig. 24.1**
**Phylogenetic reconstruction of the family *Picrophilaceae* based on 16S rRNA and created using the maximum likelihood algorithm RAxML (Stamatakis 2006). The sequence datasets and alignments were used according to the All-Species Living Tree Project (LTP) database (Yarza et al. 2010; http://www.arb-silva.de/projects/living-tree). Scale bar indicates estimated sequence divergence**

value of *Picrophilus* would in theory point to possible unusual intracellular macromolecules (e.g., differences in amino acid compositions, amino acid sequence, or three dimensional structures such as charge or hydrophobicity distributions) that are resistant and active at low pH. Both proteomic analysis and comparative proteome analysis between acidophiles and neutrophiles did not reveal any significant differences in amino acid composition or isoelectric point distributions. The only possible difference was the observation that the isoleucine content in amino acids was slightly elevated over the mean, suggesting that this may contribute to protein stability in acid (Futterer et al. 2004). Proteomic analysis of cells grown under two different pH conditions and different temperatures pointed to some upregulated proteins associated with dealing with stress, but no further insights into protein stability under low pH were revealed (Thürmer et al. 2011).

*P. torridus*'s aerobic lifestyle helps generate a substantial amount of energy that is required to maintain the huge proton gradient across the cell membrane. The organism metabolizes glucose via a nonphosphorylated Entner-Doudoroff (ED) pathway that is commonly used by thermoacidophilic Archaea. With the exception of fructose-1,6-bisphosphate aldolase, all genes for the Embden-Meyerhof-Parnas (EMP) pathway are present in *P. torridus*, and it also contains all the genes for the oxidative tricarboxylic acid cycle (TCA) (Futterer et al. 2004).

There is considerable evidence for possible lateral gene transfer within the genome of *P. torridus*. Not only does *P. torridus* share about 60 % of its homologous genes with the thermoacidophiles *T. acidophilum* and *Sulfolobus solfataricus*, 13.5 % of *P. torridus* ORFs have homologies with *S. solfataricus* but not *T. acidophilum* genomes. This suggested that these latter genes were transferred relatively recently between *P. torridus* and *S. solfataricus.*

## Phenotypic Analyses

*Picrophilus* can grow at pH and temperatures between pH 0 and 3.5 (optimum pH 0.70) and 45 °C to 65 °C (optimum 60 °C) (Schleper et al. 1995a, 1996). The organism grows aerobically on yeast extract and sugars such as glucose, sucrose, and lactose. Poor growth occurs on tryptone and growth is inhibited by 0.2 M sodium chloride. The isolates can be grown on liquid or solid media, and when grown on 12.5 % starch agar plates, the colonies are convex, whitish yellow, and 2–5 mm in diameter. Cells are nonmotile irregular cocci about 1–1.5 μm in diameter and do not have pili.

The mol% G+C of *P. oshimae* is 36 (from HPLC) and for *P. torridus* it is 35.9 (from genome sequence). Like all Archaea, both species have tetraether lipids in their membranes, which are similar in structure to *Thermoplasma* spp. Unlike *Thermoplasma*, the lipids are dominated by a beta-glucosyl resdiue (Schleper et al. 1995a). The isolates also differ from the other Thermoplasmatales as they have a distinctive 40 nm tetragonal S-layer. In contrast to other thermoacidophiles who actively maintain their internal pH at near neutral values, *Picrophilus* is able to maintain very low internal pH values (∼pH 4.6), and the membrane of *P. torridus* shows very low proton permeability and high acid stability. The membrane looses integrity if incubated above pH 4.

## Isolation, Enrichment, and Maintenance Procedures

Enrichment and growth of *Picrophilus* can be accomplished in a base *Sulfolobus* medium with 0.2 % yeast extract added as the carbon source. The base medium (Smith et al. 1975) is diluted sulfuric acid (300 ml of 0.5 M $H_2SO_4$ in 700 ml of distilled water, pH 1.0). Cultures should be incubated between 55 °C and 60 °C with shaking. Isolation of pure strains can be accomplished using 12.5 % starch plates containing the same medium. The organism can be maintained by resuspending cells in pH4.6 medium containing 20 % glycerol and stored at −70 °C.

## Ecology

These extremely acidophilic thermophiles have only been isolated from solfataras, dry acid hot soils, and sulfurous deposits in thermal springs in Hokkaido, Northern Japan. However, it is likely they could be present in other terrestrial solfataras

(Reysenbach 2001), but because of their extreme culturing conditions, they have escaped additional isolations. No culture-independent diversity assessments have detected this lineage in acidic samples yet.

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