

Draft Genome Sequence of *Pyrodictium occultum* PL19^T, a Marine Hyperthermophilic Species of *Archaea* That Grows Optimally at 105°C

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We report here the draft genome sequence of *Pyrodictium occultum* PL19^T, a marine hyperthermophilic archaeon. The genome provides insights into molecular and cellular adaptation mechanisms to life in extreme environments and the evolution of early organisms on Earth.

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Pyrodictium occultum PL19^T, a member of the order *Desulfurococcales* (phylum *Crenarchaeota*, kingdom *Archaea*), was the first hyperthermophile cultured in the laboratory at temperatures above the boiling point of water (1). Isolated from a shallow submarine solfataric field near the Volcano Island (Italy), *P. occultum* has an optimum growth temperature of 105°C and is an obligate chemoautotrophic anaerobe, which fixes CO₂ using energy derived from sulfur reduction by hydrogen (2). These characteristics represent adaptations to physical and chemical conditions associated with early Earth environments and may indicate that such organisms are ancient survivors from billions of years ago (3). Life at such high temperatures poses unique biochemical adaptation challenges in terms of macromolecular stabilization and biosynthesis of heat-labile metabolites. *P. occultum* and subsequently isolated related species were used for some of the first molecular characterizations of such adaptations, including the presence of abundant specialized chaperones, nucleotide modifications, and enzymatic activities of individually isolated proteins and energy-generating membrane-associated complexes at boiling temperatures (4–10).

P. occultum PL19^T was cultured anaerobically at 105°C in artificial seawater medium, with H₂ as the energy source (2), at the Archäozentrum facility of the University of Regensburg (Germany). Genomic sequencing was performed using the Illumina MiSeq platform, followed by quality-based read trimming, as described previously (11), which generated 22.5 million paired-end reads, with an average length of 276 bp. After evaluating several approaches (12), the optimal assembly statistics were obtained using the SPAdes software (version 3.1.0) (13) and consisted of 14 large (≥500 bp) contigs. After additional read mapping to extend the contig ends and superassembly with Geneious (version 8.1) (14), we obtained a final assembly composed of 2 contigs, with a total genome size of 1.6 Mbp. Gene prediction and annotation were performed using NCBI Prokaryotic Genome Automatic Annotation Pipeline (PGAAP) (15). The draft genome has an overall G+C content of 63.4%, a largest contig of 1.5 Mbp, and 3,360

candidate protein-coding genes. Genome integrity was confirmed by the CheckM tool (16), which estimated 98.1% completeness based on presence of 245 marker genes.

A unique characteristic of *Pyrodictium* species is the formation of an extracellular network of thin filaments, tens of micrometers long, which connect many cells in millimeter-sized mycelium-like aggregates (1, 17). The filaments are composed of hollow ultrathin (~30-nm diameter) tubes (cannulae) that penetrate the periplasmic space of individual cells and assemble from at least three related proteins, characterized biochemically from *Pyrococcus abyssi* (18, 19). The biological function of those tubes has remained unknown for decades, and no sequence or structural homologues of the cannula proteins can be identified in any other organisms. In the genome of *P. occultum*, we identified five genes likely encoding cannula proteins (CF15_01655, CF15_06945, CF15_06965, CF15_07030, and CF15_07065). The availability of the *P. occultum* genome sequence should further enable evolutionary, physiological, and molecular investigations of these hyperthermophilic archaea.

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession no. [LNTB00000000](https://www.ncbi.nlm.nih.gov/nuclink/LNTB00000000). The version described in this paper is version LNTB01000000.

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