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Pyrolobus fumarii, gen. and sp. nov., represents a novel group of archaea, extending the upper temperature limit for life to 113°C

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Abstract A novel, irregular, coccoid-shaped archaeum was isolated from a hydrothermally heated black smoker wall at the TAG site at the Mid Atlantic Ridge (depth 3650 meters). It grew at between 90°C and 113°C (optimum 106°C) and pH 4.0–6.5 (optimum 5.5) and 1%–4% salt (optimum 1.7%). The organism was a facultatively aerobic obligate chemolithoautotroph gaining energy by H_2-oxidation. Nitrate, $S_2O_3^{2-}$, and low concentrations of O_2 (up to 0.3% v/v) served as electron acceptors, yielding NH_4^+, H_2S, and H_2O as end products, respectively. Growth was inhibited by acetate, pyruvate, glucose, starch, or sulfur. The new isolate was able to form colonies on plates (at 102°C) and to grow at a pressure of 25000 kPa (250 bar). Exponentially growing cultures survived a one-hour autoclaving at 121°C. The GC content was 53 mol%. The core lipids consisted of glycerol – dialkyl glycerol tetraethers and traces of 2,3-di-*O*-phytanyl-*sn*-glycerol. The cell wall was composed of a surface layer of tetrameric protein complexes arranged on a p4-lattice (center-to-center distance 18.5 nm). By its 16S rRNA sequence, the new isolate belonged to the *Pyrodictiaceae*. Based on its GC-content, DNA homology, S-layer composition, and metabolism, we describe here a new genus, which we name *Pyrolobus* (the "fire lobe"). The type species is *Pyrolobus fumarii* (type strain 1A; DSM 11204).

Key words Archaea · Hyperthermophilic · Temperature · Hydrothermal · Taxonomy · Marine

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Introduction

The upper temperature limit for life is found among the most extreme members of hyperthermophiles known so far (Blöchl et al. 1995; Stetter 1996). They belong to the archaeal genera *Pyrodictium* and *Methanopyrus*, growing (in conditions of slight overpressure) at temperatures of up to 110°C (Stetter 1982; Stetter et al. 1983; Pley et al. 1991; Huber et al. 1989a; Kurr et al. 1991). Cultures of *Pyrodictium* consist of ultraflat disk-shaped cells connected by hollow tubules (König et al. 1988; Rieger et al. 1995) which grow strictly anaerobically by reduction of sulfur compounds (Stetter 1996). *Methanopyrus* is a rod-shaped methanogen, growing strictly autotrophically by CO_2 reduction and harboring a unique, possibly primitive lipid (Huber et al. 1989a; Kurr et al. 1991; Hafenbradl et al. 1993; Hafenbradl et al. 1996). So far, the upper limit of growth temperature is unknown and may be determined by rapid destruction of cell components (Bernhardt et al. 1984; Trent et al. 1984). Here, we describe a novel submarine hyperthermophile that extends the upper growth temperature of life and that exhibits unprecedented metabolic and physiological properties.

Materials and methods

Collection of samples

Rock samples from the walls of active "black smoker" chimneys were taken by the research submersible *Alvin* at the Mid Atlantic Ridge (TAG site: 26° N, 45° W, depth 3650 m), at the Guaymas basin (27° N, 111° W, depth 2000 m), and at the East Pacific Rise (21° N, 109° W, depth 2500 m). Samples were placed in boxes that could be closed with a tight lid mounted on *Alvin*'s working platform. In the ship's laboratory, samples of rock material were placed in 100-ml storage bottles, which then were filled with "box water." After the bottles were tightly stoppered, resazurin was injected, followed by a 10% Na_2S solution, until the resazurin became

colorless. The sample bottles were brought back to the laboratory at ambient temperature.

Strains and culture conditions

Pyrodictium occultum DSM 2709 and *Pyrodictium abyssi* DSM 6158 were from the culture collection of our institute and were cultivated in "$\frac{1}{2}$ SME-medium" as described (Stetter 1982; Stetter et al. 1983; Pley et al. 1991). Isolate 1A, if not mentioned otherwise, was grown in modified "$\frac{1}{2}$ SME-medium" ("$\frac{1}{2}$ SME*-medium") with the following composition (mg/l): $NaNO_3$ (1000); NaCl (13860); $MgSO_4 \cdot 7H_2O$ (3530); $MgCl_2 \cdot 6H_2O$ (2750); $CaCl_2 \cdot 2H_2O$ (751); KH_2PO_4 (500); KCl (325); NaBr (50); H_3BO_3 (15); $SrCl_2 \cdot 6H_2O$ (7.5); $MnSO_4 \cdot 2H_2O$ (5); $(NH_4)_2$ $Ni(SO_4)_2$ (2); $CoCl_2 \cdot 6H_2O$ (1); $FeSO_4 \cdot 7H_2O$ (2); $ZnSO_4$ (1); $KAl(SO_4)_2$ (0.1); $Na_2MoO_4 \cdot 2H_2O$ (0.1); $Na_2WO_4 \cdot 2H_2O$ (0.1); Na_2SeO_4 (0.1); $CuSO_4 \cdot 5H_2O$ (0.1); KI (0.025); resazurin (1); H_2O bidest (1 liter). After solubilization, the medium was flushed with N_2 for 30 min (11 N_2/min). Then, $Na_2S \cdot 9H_2O$ (200 mg) was added and the pH was adjusted to 5.5 (H_2SO_4). Twenty-ml portions of the medium were placed into 100-ml serum bottles which were then tightly stoppered. As the gas phase for cultivation, H_2/CO_2 (300 kPa; 80:20 v/v) was used routinely. The medium was autoclaved (200 kPa, 20 min, 121°C). Liquid cultures were incubated in hot air incubators or fermentors at 106°C under shaking or stirring (150 rpm). Mass cultures (2% v/v inoculum) were grown at 106°C in a novel 340-l enamel titanium fermentor equipped with a gas recirculation system and pH control (HNO_3). The gassing rate was 300 l/min (H_2/CO_2 80:20, 300 kPa). Microaerobic media did not contain resazurin or sodium sulfide. In this case, solubilized salts were gassed for 45 min with N_2 (1 l/min). The medium was then heated to 65°C (15 min), evacuated three times, and finally pressurized by N_2 (200 kPa). For plating, $\frac{1}{2}$ SME*-medium was solidified by 0.8% Gelrite (Kelco, San Diego, CA, USA). The plates were incubated at 102°C in a pressure cylinder (Balch et al. 1979) containing an atmosphere of H_2/CO_2 of 80:20 (200 kPa). In addition, 2% H_2S was injected into the gas phase. The salt-dependence of growth was determined by varying major salt components (NaCl; $MgSO_4 \cdot 7H_2O$; $MgCl_2 \cdot$ $6H_2O$; $CaCl_2 \cdot 2H_2O$; KCl; KH_2PO_4) by the same ratio indicated for NaCl.

Determination of growth temperatures

In order to determine the temperature-dependence of growth, the culture bottles were completely immersed in glycerol incubators and the temperatures determined using a calibrated thermometer.

Light and electron microscopy

Light microscopy was carried out as described by Huber et al. (1989b). Growth was determined by direct cell counting using a Thoma-chamber (depth 0.02 mm; Brand, Wertheim, Germany). Electron microscopy was carried out as described by Huber et al. (1996).

High pressure incubator

Incubation at high pressure was carried out within a glass syringe equipped with a closed outlet and a free-floating piston. For growth experiments, the syringe was placed into a pressurizable hydraulic chamber within a heating jacket (temperature adjustable up to 150°C).

Analyses of substrates and metabolic products

Nitrate, nitrite, NH_3, and H_2S were analyzed as described previously (Huber et al. 1992). N_2 and N_2O were determined by gas chromatography (Völkl et al. 1993).

DNA isolation and base composition

DNA was prepared as described by Lauerer et al. (1986). The G + C content was determined by melting point analysis (Marmur and Doty 1962) using calf thymus DNA (42 mol% GC) as a reference. Direct analysis of the DNA base composition was performed by high performance liquid chromatography (HPLC) after digestion of the DNA with nuclease P1 (Zillig et al. 1980; Völkl et al. 1993).

DNA/DNA hybridization

DNA/DNA hybridization was performed on minifilters (Denhardt 1966) as described by Pley et al. (1991).

Lipid analyses

Lipids were extracted from freeze-dried cells (200 mg) and analyzed (De Rosa et al. 1983; Trincone et al. 1988; Völkl et al. 1993). In addition, nonhydrolyzed apolar lipids were extracted according to Hafenbradl et al. (1993) and analyzed by thin layer chromatography (TLC) using the solvent *n*-hexane/ethyl acetate 4:1 (v/v). The compounds were detected by spraying with anisaldehyde, followed by heating at 150°C for 5 min.

Determination of deuterium oxidation

Serum bottles (120 ml) were filled with D_2 (Linde, Höllriegelskreuth, Germany) and sealed with stoppers. Then, 20 ml of microaerobic culture medium, 60 ml of CO_2, and 1.4 ml of air were injected and the bottles autoclaved. The culture medium was then inoculated (2.5% v/v inoculum) with an exponentially growing microaerophilic culture (H_2/CO_2 80:20, 0.3% O_2). After two days of incubation at 106°C, the culture grown in the presence of D_2 was harvested by centrifugation (8000 rpm, 15 min, rotor JA20, Beckman J2-21 centrifuge Fullerton, CA, USA). Due to the rapid exchange reaction between the D_2O formed and water ($D_2O + H_2O \rightarrow 2HDO$), the culture medium contained HDO. The HDO content of the supernatant was determined by nuclear magnetic resonance (NMR) spectroscopy

(spectrometer MSL 300; Bruker, Rheinstetten, Germany). As a control, an uninoculated culture medium was treated similarly.

Results

Enrichment and isolation

For enrichment of chemolithoautotrophic nitrate-reducing hyperthermophiles, serum bottles containing 20 ml of anaerobic $\frac{1}{2}$ SME* medium (gas phase H_2/CO_2) were inoculated with about 1 g of "black smoker" rock samples. After two days of incubation at 110°C, one out of two samples (No. 1) taken at the TAG site, Mid Atlantic Ridge, gave rise to a positive enrichment culture consisting of coccoid cells which could be successfully transferred into fresh medium. The enrichment culture was purified by serial dilutions followed by plating on anaerobic $\frac{1}{2}$ SME* medium solidified by Gelrite. After seven days of incubation at 102°C, white colonies about 1 mm in diameter had developed. Plating was repeated twice. From a single colony, a liquid culture was grown ("1A"; A = "autotrophic") which served as an inoculum for all further experiments.

Five samples from the Guaymas Basin and three from the East Pacific Rise hot vents gave rise to cultures of similar-looking organisms under the same conditions. These have not yet been studied in detail.

Morphology

Cells of isolate 1A are regularly to irregularly lobe-shaped cocci, about 0.7–2.5 µm in diameter, arranged singly and in aggregates of up to three individuals (Fig. 1). Electron microscopy of ultrathin sections revealed that the cell envelope is composed of a cytoplasmic membrane, a periplasmic space (45 nm wide; Fig. 2a,b), and an S-layer. In freeze-substituted cells, the periplasmic space is stained throughout (Fig. 2a). After conventional dehydration at room temperature (Fig. 2b), only a 20-nm thick layer of the periplasmic space on top of the cytoplasmic membrane is densely stained; in the upper part, long spacer molecules become visible, presumably anchoring the S-layer molecules into the cytoplasmic membrane (arrowhead; Baumeister and Lembcke 1992). As shown by relief reconstruction (Fig. 4) of freeze-etched cells (Fig. 3), the S-layer consists of a two-dimensional crystal of tetrameric protein complexes with a central depression, most likely a pore. The complexes are arranged on a lattice with p4 symmetry and a center-to-center spacing of 18.5 nm. Often, cell–cell contact sites were visible, with the S-layer subunits of the two cells opposing each other (Fig. 2b, arrows).

Metabolism

By its energy-yielding metabolism, isolate 1A was an obligate hydrogen-dependent chemolithoautotroph. Dependent on the electron acceptor, three different metabolic types were evident:

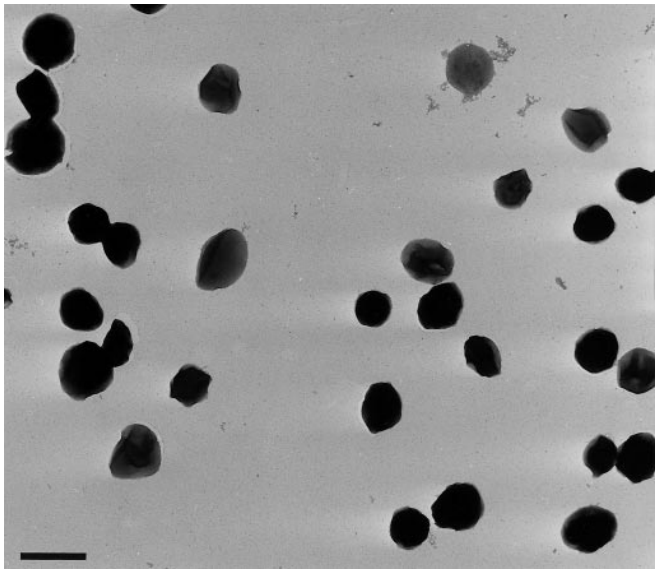

Fig. 1. Low-magnification electron micrograph of cells from isolate 1A, stained with uranyl acetate. *Bar*, 2 µm

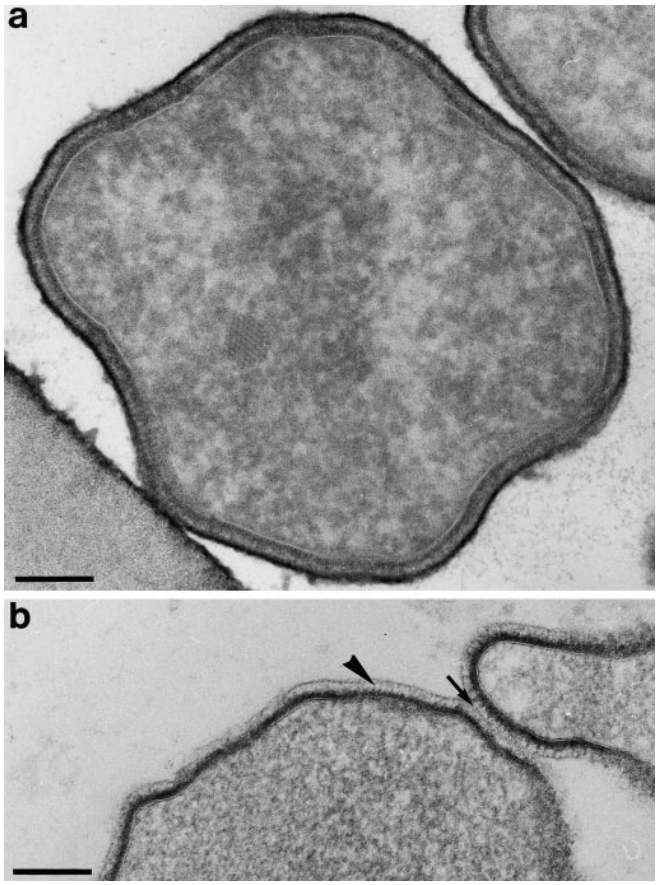

Fig. 2. Electron micrographs of ultrathin sections of cells of isolate 1A. *Bar*, 200 nm. **a** Section after freeze-substitution and Epon embedding (*bottom left*: part of the wall of the cellulose capillary tubes used for embedding). **b** Section after dehydration at room temperature and Epon embedding. *Arrowhead* points to spacer molecules in the periplasmic space. *Arrow* points to cell–cell contact sites

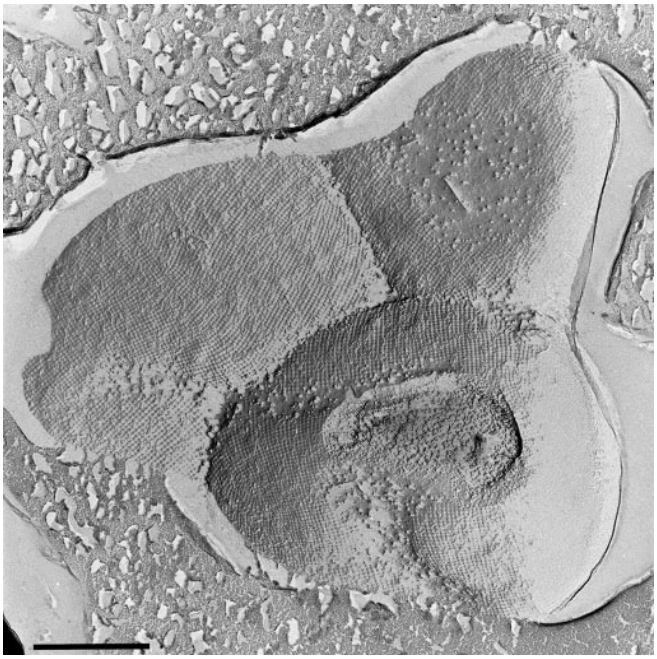

Fig. 3. Electron micrograph of freeze-etched cells of isolate 1A. *Bar*, 0.5 µm

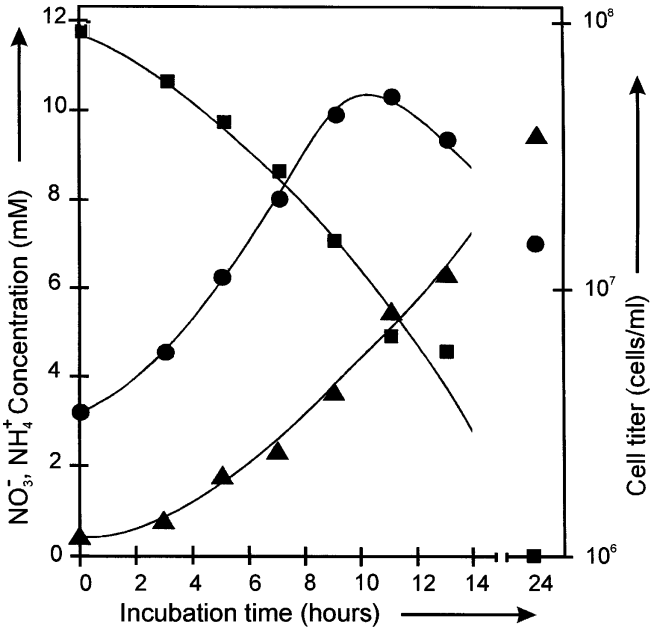

Fig. 5. Nitrate consumption and ammonia formation during growth of isolate 1A. *Circles* Growth curve, *squares* nitrate consumption, *triangles* ammonia formation

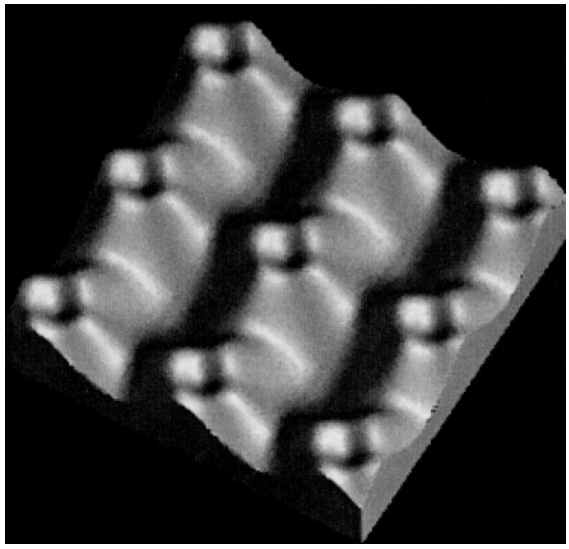

Fig. 4. Reconstruction of the surface relief of a freeze-etched cell of isolate 1A. The center-to-center distance of adjacent protein complexes is 18.5 nm

1. Nitrate ammonification

Under strictly anaerobic conditions, in the presence of NO_3^- and H_2, isolate 1A showed vigorous growth using nitrate as the terminal electron acceptor (Fig. 5). Nitrate was reduced to ammonia which accumulated within the culture medium. During growth, no detectable amounts of NO, NO_2, N_2O, or N_2 could be observed. However, during a few large-scale fermentations, NO_2^- had been formed for unknown reasons, turning colorless resazurin red (not shown). About four µmol H_2 were consumed to reduce one µmol NO_3^- (data not shown), indicating the following energy-yielding reaction:

$$4H_2 + H^+ + NO_3^- \rightarrow NH_4^+ + OH^- + 2H_2O$$

Due to ammonia formation at the expense of nitrate, at uncontrolled pH the medium quickly turned alkaline (up to pH 8).

2. Thiosulfate reduction

In the presence of H_2, in strictly anaerobic $\frac{1}{2}$ SME* medium modified by replacing $NaNO_3$ by $Na_2S_2O_3$ (0.1% w/v), the new isolate grew by thiosulfate reduction, forming H_2S (about 400 µmoles/10^8 cells). Growth was stimulated fivefold by the addition of 10 mM NH_4Cl to the medium.

3. Microaerophilic hydrogen oxidation

Isolate 1A could be adapted to grow by aerobic hydrogen oxidation at very low oxygen concentrations. Cultures growing strictly anaerobically on NO_3^- were diluted stepwise with microaerobic medium ($\frac{1}{2}, \frac{1}{4}, \frac{1}{8}, \frac{1}{16}, \ldots$) and further incubated at 106°C. Simultaneously, low concentrations of oxygen (e.g., 0.05%) were introduced into the gas phase of the culture bottles. During further transfers into nonreduced media, the O_2 content of the gas phase was stepwise increased to 0.3% (v/v). The O_2-adapted cultures could be successfully transferred into fresh media. Under these conditions (absence of NO_3^- and $S_2O_3^{2-}$), growth was strictly dependent on the presence of H_2 and O_2. In

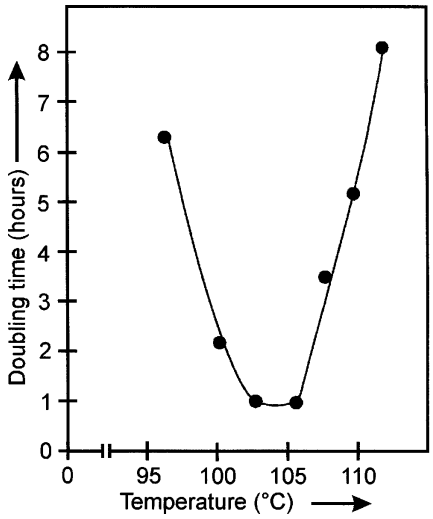

Fig. 6. Optimal growth temperature of isolate 1A during anaerobic growth on H_2 and NO_3^-. Doubling times were calculated from the slopes of the growth curves (not shown)

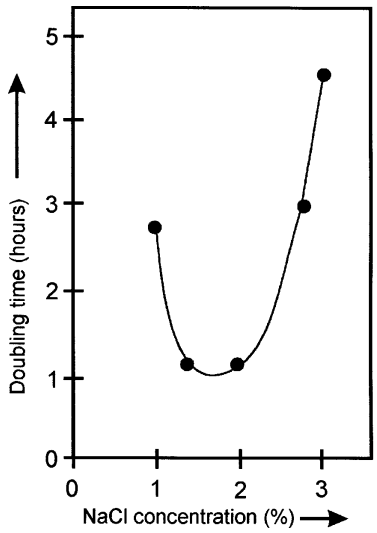

Fig. 7. Influence of NaCl on anaerobic growth of isolate 1A on H_2 and NO_3^-. Doubling times were calculated as for Fig. 6

order to confirm aerobic H_2 oxidation independently, a culture of isolate 1A was grown microaerobically with deuterium as the electron donor. After growth for one day, NMR spectroscopic analysis revealed the formation of 18 μmoles $D_2O/10^7$ cells, indicating deuterium oxidation.

Physiological characterization

Isolate 1A grew between 90°C and 113°C with an optimum at around 106°C (1-hour doubling time; Fig. 6). At 90°C, the doubling time was 36 h (not shown). No growth could be observed at 85°C or at 115°C. The pH range of growth was between pH 4.0 and 6.5 (optimum 5.5; not shown). The isolate grew in NaCl at concentrations between 1% and 4% with an optimum at 1.7% (Fig. 7). Cells lysed at 0.5% NaCl or below. When elemental sulfur, S°, was added into exponentially growing cultures (0.02% w/v and more), cells lysed within one hour. On nitrate and thiosulfate in the presence of 0.1% (v/v) O_2, growth of isolate 1A was completely inhibited. If oxygen (0.1% v/v) was added to exponentially growing anaerobic cultures, the number of viable cells was lowered by two orders of magnitude within 30 min (not shown). In the presence of H_2 (and nitrate or thiosulfate), supplementation with yeast extract, meat extract, peptone, tryptone, casein, gelatine, casamino acids, raffinose, or formate did not influence growth, while acetate, citrate, pyruvate, glucose, or starch strongly inhibited or even prevented growth (at 0.1% w/v). In the absence of H_2, with the same organic substances, no growth was observed.

Long-term viability and storage

When exponentially growing cultures of isolate 1A were cooled down and stored at room temperature, they could serve as inocula for more than 6 months. Long-term stock cultures, containing 5% dimethylsulfoxide, were stored in 20-μl portions under liquid nitrogen.

Survival during autoclaving

An exponentially growing (middle log phase) culture and a culture in the stationary phase of isolate 1A were autoclaved in parallel for one hour at 121°C. Microscopic inspection afterwards revealed that almost all of the stationary phase cells were hyaline, while about 1% of the exponential cells still exhibited a strong phase contrast. Accordingly, when used as an inoculum (1% v/v) in fresh medium, autoclaved exponential phase cells grew up within three days, while nonautoclaved controls grew up within one day (data not shown). This indicates that a significant number of cells in the exponentially growing culture survived autoclaving.

Growth at high pressure

Isolate 1A was grown at an in situ pressure of 25000 kPa in a novel high-pressure incubator. After 22 h, about 2×10^8 coccoid cells per ml became visible. In addition, ammonia was produced and the pH of the medium had risen from 5.5 to 7.5. A parallel culture at normal pressure (300 kPa) yielded similar results (not shown).

Hydrogenase activity

Crude extracts from cells of isolate 1A grown on H_2/NO_3^- exhibited hydrogenase activity (optimal reaction temperature at 119°C). This activity was membrane-associated and used methylene-blue as an artificial electron acceptor (data not shown).

Table 1. DNA/DNA homology of *Pyrodictium* species and the new isolate 1A (in %)

Source filter-bound DNA	Source of ^{32}P-labeled DNA		
	P. occultum (DSM 2709)	*P. abyssi* (DSM 6158)	Isolate 1A
P. occultum (DSM 2709)	100	12	3
P. abyssi (DSM 6158)	25	100	7
Isolate 1A	10	8	100
Calf thymus (control)	8	7	3

Presence of a heat shock protein

By immunoblotting, crude extracts of cells of isolate 1A showed a strongly positive cross-reaction with antibodies prepared against the thermosome of *Pyrodictium occultum* (Phipps et al. 1991). This was used as evidence that isolate 1A harbored a chaperone-like heat shock protein.

Lipid composition

The complex lipid pattern of isolate 1A, as analyzed by TLC, consisted of two major spots of glycophospholipids at R_f 0.10 and 0.20 and one spot of a phospholipid at R_f 0.4. Hydrolysis of complex lipids yielded uncyclized glycerol-dialkyl-glycerol-tetraether (GDGT) as the major compound and traces of 2,3-di-*O*-phytanyl-*sn*-glycerol (diether). C_{20} C_{25} diethers and cyclized GDGT were absent. Analysis of nonhydrolyzed lipids revealed a so far uncharacterized main spot (R_f 0.4) which stained blue with anisaldehyde (instead of violet as would be characteristic for a nonpolar lipid). Further investigation of this novel compound is in progress.

DNA base composition

The GC content of the DNA of isolate 1A was 52.9 mol%, as determined by direct analysis of the mononucleosides, and 53.4 mol% by melting point analysis.

DNA–DNA homology

By 16S rRNA partial sequencing, members of *Pyrodictium* are the closest relatives of isolate 1A (Burggraf et al., 1996 unpublished work). In a DNA/DNA hybridization experiment, isolate 1A exhibited only insignificant DNA homology with the type species *Pyrodictium occultum* and *Pyrodictium abyssi* (3% to 10%; Table 1).

Discussion

The novel isolate 1A represents the most extreme hyperthermophile known so far. By thriving within the temperature range of 95°C–113°C, it extends our knowledge about the upper temperature limit for life (Stetter 1995; Stetter 1996). It is remarkable that a significant proportion of exponentially growing cells of the new isolate survived one hour of autoclaving at 121°C. A similar observation had been made recently for *Pyrodictium occultum* (Stetter et al. 1993). Considering the lowest growth temperature of isolate 1A, it is equally remarkable that, for the first time, a hyperthermophilic organism is unable to grow at pasteurization temperatures of 85°C. The temperature range of growth of the new isolate appears surprisingly narrow (about 20°C) if compared with that of other organisms (including hyperthermophiles; Schlegel and Jannasch 1992; Stetter 1994). Through an improvement of culture conditions, it might be possible to increase the upper limit of the growth temperature beyond 113°C (e.g., 120°C–125°C). On the other hand, in view of rapid heat destruction of cell components, 113°C may already be the highest growth temperature possible (Bernhardt et al. 1984).

According to its 16S rRNA sequence and the presence of phytanyl ether lipids, isolate 1A is a member of the archaeal domain (Woese and Fox 1977; Woese et al. 1990; Langworthy et al. 1982). On the 16S rRNA-based phylogenetic tree, it belongs to the *Pyrodictiaceae* lineage, which is one of the deepest and shortest branches close to the root, suggesting that its members may still be rather primitive (Burggraf, Huber, and Stetter, submitted). Therefore, the high growth temperatures of the novel isolate in combination with its phylogenetic position further support the assumption of a hyperthermophilic common ancestor of life (Stetter 1992). Compared to members of *Pyrodictium*, isolate 1A shows significant differences in morphological, physiological, and biochemical features by its (a) lack of extracellular tubules and their characteristic networks, (b) tetragonal instead of hexagonal array of the S-layer, (c) novel nonpolar lipid, (d) nitrate and oxygen respiration, (e) inhibition of growth by elemental sulfur and several organic nutrients, (f) strictly autotrophic mode of life, (g) 9 mol% lower GC-content, (h) insignificant DNA/DNA homology, and (i) different 16S rRNA sequence (Burggraf, Huber, and Stetter, submitted). Therefore, isolate 1A represents a novel genus which we name *Pyrolobus* ("the fire lobe"). The type species is *Pyrolobus fumarii* ("the fire lobe of the chimney"), considering the habitat from which it was isolated. The type strain is *Pyrolobus fumarii* 1A (DSM 11204).

Pyrolobus fumarii is well adapted to its deep-sea vent environment by its resistance to high pressure, its salt requirement, and its high growth temperature. Using CO_2 as the single carbon source and H_2 as the obligate electron donor in its energy-yielding reactions, this organism carries out the primary production of organic matter at the deep-sea hydrothermal vents.

Both CO_2 and H_2 are commonly found in hydrothermal fluids, as well as the electron acceptors nitrate and thiosulfate (Corliss et al. 1979; Jannasch and Mottl 1985). Similar to *Pyrobaculum aerophilum*, a denitrifying hyperthermophile from shallow marine vents (Völkl et al. 1993), *Pyrolobus fumarii* is able to gain energy by hydrogen oxida-

tion at very low oxygen concentrations. In the largely reducing environment within the walls of hot smokers, the source of low-level oxygen is so far unknown. The hydrothermal venting system might entrain oxygen-rich deep-sea water into the porous black smoker walls, making traces of free oxygen available to *Pyrolobus fumarii*. It appears surprising that members of the deepest and shortest branches within the phylogenetic tree such as *Aquifex pyrophilus*, *Pyrobaculum aerophilum*, and the newly isolated *Pyrolobus fumarii* are facultative microaerophiles (Huber et al. 1992; Völkl et al. 1993), since these organisms are considered as rather primitive (Stetter 1994). Free oxygen is assumed to have appeared globally rather late within Earth's atmosphere (Schopf et al. 1983). However, traces of oxygen could already have been formed photochemically early in the history of the Earth, and therefore could have been used by organisms existing at that time (Towe 1988; Towe 1994). On the other hand, hyperthermophilic microaerophiles are powerful nitrate reducers, and could have conducted a kind of "precursor metabolism." Nitrate might have formed from N_2 and CO_2 as a result of lightning within a primitive atmosphere (Mancinelli and McKay 1988). With their tremendous heat-resistance, *Pyrolobus*-like organisms could have even survived heat shocks created by the heavy impacts to which the Earth was exposed during Hadean times (Sleep et al. 1989). Because they require only the basic nutrients generated by volcanism, these organisms would be able to exist on any planet that possessed volcanic activity and liquid water.

Description of *Pyrolobus* gen. nov.

Pyrolobus (Pyr.o.lo' bus Gr. neutr. n. *pyr*. fire; Gr. masc. n. *lobos*. lobe; M.L. masc. n. *Pyrolobus* the "fire lobe"). Gram-negative immotile regularly to irregularly lobed cocci, occurring singly and in short chains. No spores formed. S-layer cell envelope of protein subunits arranged on a p4 lattice. Membrane contains glycerol-dialkyl-glycerol-tetraether. No murein present. Growth at 4% (w/v) NaCl and up to 113°C. No growth at 85°C and below. Facultatively aerobic. Obligately chemolithoautotrophic. Growth with H_2 by reduction of NO_3^-, thiosulfate, and O_2. DNA base composition 53 mol% GC. By 16S rRNA sequence, specifically related to the *Pyrodictiaceae.* Habitat: Abyssal deep-sea hydrothermal systems.
Type species: *Pyrolobus fumarii*

Description of *Pyrolobus fumarii* sp. nov.

Pyrolobus fumarii (fum.a' ri.i L. gen. n. *fumarii* of the chimney, referring to its black smoker biotope). Cells regularly to irregularly lobed cocci, about 0.7 to 2.5 µm in diameter. White colonies, about 1 mm in diameter, formed on plates solidified by Gelrite. Growth between 90°C and 113°C (optimum 106°C), at pH 4.0–6.5 (optimum pH 5.5), and at 1%–4% NaCl (optimum: 1.7%). Pressure of 25000 kPa tolerated. Exponential cultures survive a 1-hour autoclaving at 121°C. Optimal doubling time, 60 min. Strictly chemolithoautotrophic growth by anaerobic and microaerophilic H_2 oxidation, with NO_3^-, $S_2O_3^{2-}$, and O_2 as electron acceptors. NO_3^- is reduced to ammonia. No stimulation of growth by organic compounds. Growth inhibition by acetate, pyruvate, glucose, starch, or elemental sulfur. Cell envelope composed of a protein surface layer, exhibiting p4 symmetry and a center-to-center spacing of neighboring protein complexes of 18.5 nm. Core lipids mainly glycerol-dialkyl-glycerol-tetraether. Non-hydrolyzed lipids contain a main spot on TLC staining blue (instead of violet) by anisaldehyde. DNA base composition, 53 mol% GC. Type strain: *Pyrolobus fumarii* 1A, DSM 11204, Braunschweig, Germany (isolated from a black smoker wall, TAG site, Mid Atlantic Ridge).

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