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CHAPTER 5

The Order Thermococcales

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Among the hyperthermophilic archaea, representatives of order Thermococcales form the most numerous group to date. Members of this group are the most frequently isolated hyperthermophiles. They are heterotrophic and as such regarded as the major constituents of organic matter within marine hot water ecosystems (Canganella et al., 1997). They belong to the branch of Euryarchaeota that contains the methanogens, the genus Thermoplasma, and the extremely halophilic archaea. The Thermococcales order is actually represented by three genera: *Pyrococcus* (Fiala and Stetter, 1986), *Thermococcus* (Achenbach-Richter et al., 1988) and the newly described *Paleococcus* (Takai et al., 2000). Phylogenetic analysis based on 16 rDNA sequences indicates that the *Paleococcus* strains are members of an ancient lineage of Thermococcales that diverged prior to the formation of the genera *Pyrococcus* and *Thermococcus* (Takai et al., 2000). These three genera include at present 38 species: 2 belonging to the genus *Paleococcus*, 6 belonging to the genus *Pyrococcus*, and 30 to the genus *Thermococcus*. The optimal growth temperature is 95–100°C for members of the genus *Pyrococcus* and 80–90°C for those of the genus *Thermococcus*. *Pyrococcus* strains have been isolated only from marine hydrothermal vents, whereas species belonging to the genus *Thermococcus* have been isolated also from terrestrial fresh water (Ronimus et al., 1997), marine solfataric ecosystems, deep-sea hydrothermal vents (Stetter, 1996) and offshore oil wells (Takahata et al., 2001). Representatives of the order Thermococcales have coccoid cells with or without flagella; they are obligate anaerobic organotrophic thermophiles with a fermentative metabolism using peptides, polysaccharides, or other sugars as carbon sources. Elemental sulfur is either stimulatory or necessary for the growth of these microorganisms. Molecular hydrogen that is produced during fermentation reduces elemental sulfur to H_2S (Schönheit and Schäfer, 1995). Most of Thermococcales are neutrophiles growing optimally at pH 6.0–7.0; only the two species *Thermococcus alcaliphilus* (Keller et al., 1995) and *T. acidoaminovorans* (Dirmeier et al., 1998) are able to grow optimally at pH 9.0.

Ecology

The Thermococcales are generally found in natural biotopes that are typical for thermophilic microoganisms. They were originally discovered in terrestrial and submarine hot vents and they were then found also in deep subsurface environments. For example, *Thermococcus celer* (Zillig et al., 1983), *T. litoralis* (Britton et al., 1995) and *Pyrococcus* sp. were discovered in an offshore oil production platform in the North Sea (Stetter, 1996), and *T. litoralis* (Neuner et al., 1990) was isolated from a continental oil well (Paris Basin, France). This fact probably indicates the indigenous origin of hyperthermophilic archaea in the deep subsurface biosphere. Another strain, *T. sibiricus*, was isolated from a high temperature oil reservoir in Western Siberia, a location far remote from both the ocean and volcanic areas. The sites where these microorganisms are found can appear to be unusual, but these microorganisms might have been deposited with the original sediment and survived over geologic time by metabolizing buried organic matter (Miroshnichenko et al., 1998; Miroshnichenko et al., 2001).

On the other hand, members of the genus *Pyrococcus* seem to be isolated from only marine environments and belong to a particular ecological niche (Morikawa et al., 1994). As a result of the hydrostatic pressure at deep-sea vents, geothermally heated seawater remains liquid at temperatures up to 400°C (Stetter, 1996; Duffaud et al., 1998). When the hot fluid that is enriched in polymetal sulfides and gasses is mixed with cold (2°C) seawater, minerals precipitate and form so-called "chimneys," or "smokers" (Fiala and Stetter, 1986). The temperature of the hot fluid that is emitted from the chimney can even reach 300°C (Holden and Baross 1993; Kwak et al., 1995; Gonzalez et al., 1998; Cambon-Bonavita et al., 2003). Members of the genus *Pyrococcus* are common inhabitants of this eco-

logic environment together with other hyperthermophiles belonging to the genera of *Pyrodictium*, *Pyrobaculum*, *Pyrolobus* and *Methanopyrus*. The two strains *Pyrococcus abyssi* and *Paleococcus horikoshii* were isolated from deep-sea vents of the North Fiji Basins, South Pacific Ocean (Erauso et al., 1993), and Okinawa Trough, Japan (Gonzalez et al., 1998), respectively. *Pyrococcus furiosus* and *Pyrococcus woesei* have been discovered along the marine solfataras of the island Vulcano (Italy). The novel barophilic archaeon belonging to the genus *Palaeoococcus* was collected from a deep-sea hydrothermal vent chimney at the Myojin Knoll in the Ogasawara-Bonin-Arc, Japan (Takai et al., 2000).

Isolation

For the isolation of hyperthermophilic archaea, complex anaerobic media are usually used which are prepared according to the Hungate technique (Blamey et al., 1999). After taking samples with special syringes, they should be inoculated into serum vials (25–100 ml) or Hungate tubes (10 ml) containing media under an anaerobic atmosphere of CO_2 or N_2. The 1000 ml of medium should also contain 0.2–1% of elemental sulfur and 10–100 µl of resazurin (1 mg/ml) as a redox indicator. Before inoculation, the medium should be reduced with sodium sulfide (Na_2S; 0.05%). The sample may be transported at ambient temperature and kept at 4°C. Most Thermococcales may survive for a long time at cold or ambient temperature. Using special equipment, the samples taken from the deep sea can be transferred to the laboratory under pressure, and enrichment cultures can be prepared under high pressure and temperature. Samples from the deep sea can be collected by employing a manned submersible. In many cases and to increase the cell concentration, marine samples have to be concentrated many fold using a sterilized cross-flow device equipped with a 50-kDa cut-off membrane. For the growth and isolation of marine Thermococcales, the medium MB (Bacto Marine Broth, Difco) can be used and contains, in general, the following components per liter: bactopeptone, 5 g; bactoyeast extract, 1 g; Fe(III) citrate, 0.1 g; NaCl, 19.45 g; $MgCl_2$, 5.9 g; $NaSO_4$, 3.24 g; $CaCl_2$, 1.8 g; KCl, 0.55 g; Na_2CO_3, 0.16 g; KBr, 0.08 g; $SrCl_2$, 34 mg; H_3BO_3, 22 mg; Na-silicate, 4 mg; NaF, 2.4 mg; $(NH_4)NO_3$, 1.6 mg; and Na_2HPO_4, 8 mg; the final pH is adjusted to 7.6–7.8. If the complete medium from Difco is used, 37.4 g must be added to 1 liter of water. It is important to filter the medium using normal filter paper to prevent the possible precipitation of iron. The medium that is normally prepared for the cultivation of *Pyrococcus* species is as follows: KH_2PO_4, 0.5 g; $NiCl_2 \cdot 6H_2O$, 2 mg; trace element solution (Balch et al., 1979), 10 ml; sulfur, 30 g; yeast extract, 1 g; peptone, 5 g; and resazurin, 1 mg. The pH has to be adjusted to 6.4–6.5. After boiling the above-mentioned media under N_2 atmosphere, they should be cooled on ice and transferred under N_2 atmosphere to 10-ml Hungate tubes and sterilized by autoclaving. Before use, the medium must be reduced with a sterile neutral solution of $Na_2S \cdot 9H_2O$ (0.5 g/liter).

To obtain single colonies on plates, the same media can be used including Gelrite (0.8%), and the plates should be stored in anaerobic jars under a N_2 atmosphere at the desirable temperature.

Cultivation

Thermococcales are receiving increasing interest from academia and industry because they provide a unique source of stable biocatalysts and other products such as archaeal lipids and compatible solutes. However, until recently only low cell concentrations (10^7–10^8 cells/ml) could be obtained, making application studies very difficult. The main reason for this has to be ascribed to difficulties related to the production and purification of large quantities of biocatalysts and cell components. Special equipment is also needed to cultivate some strains under high pressure and temperature. Innovative bioreactor design to improve biomass yield is required. Because the accumulation of toxic compounds is thought to be responsible for low biomass yields, dialysis fermentation of *Paleococcus woesei* (Blamey et al., 1999) and *P. furiosus* has been performed for effective removal of low-molecular-mass components from the fermentation broth. Unlike many other heterotrophic hyperthermophiles, significant growth of *P. furiosus* is not dependent on the presence of elemental sulfur (S^0). When dialysis membrane reactors were applied, a dramatic increase in cell yields was achieved (Krahe et al., 1996). The cultivation of the hyperthermophilic archaeon *Pyrococcus furiosus* (growth at 90°C) resulted in cell yields of 2.6 $g \cdot liter^{-1}$. For *P. furiosus* the optimum stirrer speed was 1,800 rpm, and neither hydrogen nor the metabolic products were found responsible for the comparatively low cell yield. The fermentation processes can be scaled-up from 3 liters to over 30 liters (up to 300 liters). The pilot plant scale offers the possibility of transferring the fermentation performance to a larger industrial scale. In recent experiments it was shown that even the results of the 1-liter dialysis reactor can be reproduced in 30-liter reactors using

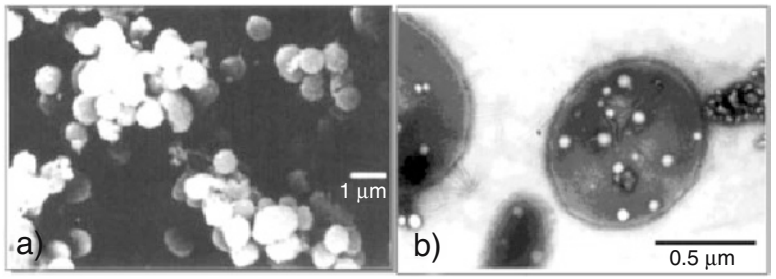

Fig. 1. Transmission electron micrographs of a) *Thermococcus aggregans* (from Canganella et al., 1998), b) *Thermococcus siculi* (from Grote et al., 1999), negatively stained with 0.3% phosphotungstic acid.

external dialysis modules. On the other hand, a method was described for growing *P. furiosus* in 600-liter fermentors (Verhagen et al., 2001), which resulted in the production of 500 g of cells (wet weight).

As already mentioned, Thermococcales, isolated from deep sea vents, are able to grow not only at high temperature but also at hydrostatic pressure around 20–30 MPa. Owing to practical difficulties, the growth and the metabolism of barophilic Thermococcales have been poorly investigated. It has been demonstrated that the upper growth temperature is extended at least 3°C when cells of *P. abyssi* are cultivated at 20 MPa. Similar higher thermotolerance and upward shift in the optimal temperature were observed also for *P. endeavori* at 22 MPa (Erauso et al., 1993). More detailed studies with *Thermococcus peptonophilus* (Canganella et al., 1997) and *T. barophilus* (Marteinsson et al., 1999) have also demonstrated that both species are barophilic. In fact, in both cases the growth rate at the optimal growth temperature was higher under in situ hydrostatic pressure than under lower pressure.

Identification and Morphology

In general, in phase contrast microscopy, all known species of Thermococcales appear as spherical cells, mostly as diploid forms constricted to various degrees owing to the duration of the division process throughout the whole generation time. When the cells of Thermococcales are compared to those of Sulfolobales and certain Thermoproteales, the Thermococcales cells are more round, slightly irregular, and their size varies from 0.5 to 2.5 µm. In the final stage of division of Thermoccoccales cells, the two daughter cells are connected by a thin string of cytoplasm enclosed by a membrane and S-layer. In general, cells show a Gram-negative reaction. Unlike all other strains, *T. aggregans* cells form chains, and cell aggregates are particularly characteristic of this strain. However, the formation of aggregates is not a general characteristic but occurs only after cultivation of the strain on yeast-tryptone extract (Canganella et al., 1998; Fig. 1a).

Electron microscopy of Thermococcales cells reveals the presence of monopolar polytrichous flagella in most of the species. For example, *P. furiosus* (Fiala and Stetter, 1986), *P. woesei* (Zillig et al., 1987), *P. abyssi* (Erauso et al., 1993), *P. horikoshii* (Gonzalez et al., 1998), *T. acidoaminovorans* (Dirmeier et al., 1998), *T. peptonophilus* (Gonzalez et al., 1995) and *T. chitinophagus* (Huber et al., 1996) are all characterized by the presence of a tuft of polar flagella. Nevertheless, this is not a rule. In other strains, the flagella have a fimbriate arrangement as observed for *T. siculi* (Grote et al., 1999; Fig. 1b). In the case of *T. alcaliphilus* (Keller et al., 1995), only a single flagellum is present and in the case of *T. sibiricus*, the flagella are completely absent (Miroshnichenko et al., 2001).

The cytoplasmic membrane (5–10 nm) of *T. chitonophagus* is covered by a bilayered cell envelope with an inner periplasmic space (15–20 nm) and an external, densely stained layer (5 nm), probably corresponding to a surface-layer protein (Huber et al., 1996). The described organization (structure) of the cytoplasmic membrane is more or less similar among the other members of Thermococcales. Thermococcales have the typical archaeal cytoplasmic membrane lipids (ether lipids), and some members also have simple diether lipids (mainly made up of one or two phospholipids) and trace amounts of tetraethers. The presence of two rare acyclic and cyclic glycerol diphytanyl tetraethers has been reported in *T. chitinophagus* (Huber et al., 1996). However, more detailed studies have been carried out on the lipid structure of *T. hydrothermalis* isolated from a deep-sea hydrothermal vent. On the basis of acid methanolysis and spectroscopic studies, the polar lipids (amounting to 4.5% [w/w] of the dry cells) included diphytanyl glycerol diethers and dibiphytanyldiglycerol tetraethers in a 45 : 55 ratio. No cyclopentane ring was present in the tetraethers. From the neutral lipids (0.4% [w/w] of the dry cells), four di- and tri-unsaturated acyclic tetraterpenoid hydrocarbons and low amounts of di- and tetraethers

(occurring in free form) were identified. All are structurally related to lycopane. The presence of these hydrocarbons provides some evidence that lycopane, widely distributed in oceans, could be derived, at least partially, from the hydrocarbons synthesized by some hyperthermophilic archaea. Analysis of the uninoculated culture medium indicates that fatty acid derivatives and some steroid and triterpenoid compounds identified in the lipidic extract of the archaea probably originate from the culture medium (Lattuati et al., 1998).

Physiology and Metabolism

Most of Thermococcales species are obligate anaerobic organothrophic thermophiles that prefer to utilize polymeric substrates like proteins and carbohydrates (preferentially oligo- and polysaccharides) as carbon and energy sources. Elemental sulfur is required in some cases for the growth and is used as an electron acceptor to remove reducing equivalents that are produced during fermentation. However, these physiological characteristics are not the rule for all members of the order Thermococcales and some differences can be observed in the three genera *Thermococcus*, *Pyrococcus* and *Paleococcus* (Selig et al., 1997; Table 1).

Species of the genus *Pyrococcus* are heterotrophic and sulfur-reducing microorganisms. Growth is observed when complex organic substrates such as yeast extract, peptone, tryptone, meat extract, and peptides are used (Fiala and Stetter, 1986). *Pyrococcus furiosus* and *P. woesei* can also grow on various carbohydrates such as starch (Biller et al., 2002), glycogen, pullulan (Blumentals et al., 1990; Costantino et al., 1990), cellobiose and pyruvate (Kengen et al., 1993). *Pyrococcus glycovorans* grows on proteinaceous substrate and different carbohydrates and, in addition, it is able to use glucose as carbon source, a feature that appears to be unique in hyperthermophiles (Barbier et al., 1999). *Pyrococcus abyssi* and *P. horikoshii* are unable to grow on carbohydrates (Erauso et al., 1993; Gonzalez et al., 1998). Unlike the results reported in the literature, in many cases some members of Thermococcales can even grow on modified media in the absence of sulfur. Members belonging to the genus *Thermococcus* appear to grow mainly on media containing complex proteinaceous substrates such as yeast extract or tryptone as the sole carbon and energy source. Some species like *Thermococcus peptonophilus*, *T. alcaliphilus* and *T. zilligii* are unable to grow on amino acid mixtures (Gonzalez et al., 1995; Keller et al., 1995). There are few reports on the successful cultivation of hyperthermophiles on defined minimal media. A minimal defined medium has been reported for the cultivation of *Thermococcus acidoaminovorans*, which uses defined amino acids as the sole energy source (Dirmeier et al., 1998). *Thermococcus aggregans* and *T. aegaeicus* strains instead are able to use carbohydrates as substrates. *Thermococcus aggregans* is able to grow on starch and maltose and *T. aegaeicus* also utilizes starch but under a N_2/CO_2 atmosphere (Canganella, et al., 1998). Significant growth on maltose and slow growth on cellobiose were observed for *T. hydrothermalis* (Godfroy et al., 1997; Gruyer et al., 2002) and *T. fumicolans* (Godfroy et al., 1996). Interestingly, *T. chitinophagus* represents the only species able to grow on chitin as a carbon source (Huber et al., 1996). With the exception of *T. stetteri* (Miroshnichenko et al., 1989), *T. profundus* (Kobayashi et al., 1994; Kwak et al., 1995) and *T. waiotapuoensis* (Gonzalez et al., 1999), *Thermococcus* strains are stimulated by addition of sulfur, but sulfur is not absolutely required (Arab et al., 2000). The recently identified archaeon *Palaeococcus* sp. possesses most of the morphological and physiological properties typical of Thermococcales. It, however, displays an absolute requirement for either elemental sulfur or ferrous iron (Fe^{2+}). The requirement for iron represents an ancient characteristic of early microbial metabolism, in light of geochemical data suggesting the properties of habitats occupied by microorganisms belonging to the order Thermococcales (Takai et al., 2000).

The generation time of the members of Thermococcales is the shortest among Archaea. The time range is between 25 min for *T. peptonophilus* and 70 min for *T. stetteri*. The doubling time of the strains belonging to the members of the genus *Pyrococcus* is around 30–35 min (Table 1).

Sugar and Peptide Degradation Pathways

As already described, many Thermococcales show heterotrophic growth on a variety of carbohydrates. This suggests that oligosaccharides with varying degrees of polymerization are transported into the cell and are subsequently hydrolyzed to glucose. Various studies have focused both on the transport of the saccharides into the cell and on the pathways that are used to degrade the glucose (Schönheit and Schäfer, 1995). For *Thermococcus litoralis*, a transport system for both maltose and trehalose has been described that probably represents an ATP-binding cassette (ABC) transporter (Horlacher et al., 1998; Xavier et al., 1999). The trehalose-maltose binding protein, TMBP, and the ATPase subunit, MalK, have been functionally expressed in *Escherichia coli* (Greller et al., 1999; Greller et al., 2001; Diederichs et al., 2000). These binding

Table 1. Morphological and physiological characteristics of strains belonging to the order Thermococcales.

Species	G+C content (mol%)	Growth temperature (°C)		pH		NaCl concentration (%)		Carbon sources	Growth on amino acids	Sulfur effects	References
		range	opt.	range	opt.	range	opt.				
Palaeococcus ferrophilus DMJ 10246(T)	53.5	60–88	83	4.0–8.0	6.0	n.d.	n.d.	Complex substrates[a]	n.d.	E	Takai et al., 2000
Pyrococcus furiosus DSM 3638	38	70–103	100	5–9	7	0.5–5	2	Complex substrates, maltose, starch, pyruvate, and casamino acids	Yes	R	Fiala and Stetter, 1986
Pyrococcus abyssi CNCMI-1302	45	67–100	96	4–8.5	6.8	0.7–5	3	Complex substrates, maltose, starch, pyruvate, and casamino acids	Yes	E	Erauso et al., 1993
Pyrococcus woesei DSM 3773	37.5	70–105	100–103	n.d.	n.d.	n.d.	3	Yeast extract, tryptone, glycogen, and gellan	Yes	E	Zillig et al., 1987
Pyrococcus kodakaraeinsis DSM 3773	38	65–100	95	5–9	7	1–5	3	Complex substrates, and peptides	Yes	E	Morikawa et al., 1994
Pyrococcus endeawori (ES4)	55	80–110	98	4–8	7.0	n.d.	2.8	Casamino acids	Yes	E	Holden and Baross, 1993
Pyrococcus horikoshii JCM 9974	44	80–102	98	5–8	7.0	1–5	2.4	Complex substrates	Yes	E	Gonzalez et al., 1998
Pyrococcus glycovorans (Al585T)	47	75–104	95	2.5–9.5	7.5	2–6	3	Complex substrates, and glucose	Yes	E	Barbier et al., 1999
Thermococcus celer DSMZ 2476	57	Up to 93	88	n.d.	5.8	n.d	4	Peptides stimulated by sucrose	Yes	E	Zillig et al., 1983
Thermococcus litoralis DSMZ 5474	38	65–95	88	6.2–8.5	7.2	1.8–6.5	2.5	Peptides, and pyruvate	No	E	Neuner et al., 1990 Britton et al., 1995
Thermococcus stetteri DSMZ 5262	50	60–85	75	5.7–7.2	6.5	1–4	2.5	Starch, pectin, and peptides	No	R	Miroshnichenko et al., 1989
Thermococcus profundus DT5432 52.2	52.2	50–90	80	4.5–8.5	7.5	1–6	2	Pyruvate, starch, and maltose	n.d.	R	Kobayashi et al., 1994
Thermococcus peptonophilus JCM 9653	52	60–100	85	4–8	6	1–5	3	Peptides	n.d.	E	Gonzalez et al., 1995
Thermococcus aggregans DSMZ 10597	42	60–94	88	4.6–7.9	7	n.d.	2	Complex substrates, dextrose, and maltose	n.d.	E	Canganella et al., 1998
Thermococcus pacificus DSMZ 10394	53.3	70–95	80/88	6–8	6.5	1–5	2–3.5	Complex substrates	n.d.	E	Miroshnichenko et al., 1998

Table 1. *Continued*

Species	G+C content (mol%)	Growth temperature (°C)		pH		NaCl concentration (%)		Carbon sources	Growth on amino acids	Sulfur effects	References
		range	opt.	range	opt.	range	opt.				
Thermococcus guaymasiensis DSMZ 11113	46	56–90	88	5.6–8.5	7.2	1–5	2–3.5	Casein, dextrose, and maltose	n.d.	E	Canganell et al., 1998
Thermococcus gorgonarius DSMZ 10395	50.6	68–95	80/88	3.4–9	7	2–10	3	Complex substrate	n.d.	E	Miroshnichenko et al., 1998
Thermococcus hydrothermalis CNCM I1319	58	53–100	85	2–10	7	Sea salt	4	Casein, peptides, and maltose	Yes	E	Godfroy et al., 1997
Thermococcus zilligii DSMZ 2770	46.2	n.d.	75	n.d.	7.4	n.d.	25	Casein	n.d.	E	Ronimus et al., 1997
Thermococcus acidaminovorans DSMZ 11906	49	56–93	85	5–9.5	9	1–6	2–3	Peptides	Yes	E	Dirmeier et al., 1998
Thermococcus aegeicus DSMZ 12767	45.5	50–90	88	4–9	6	0.5–6.5	2.7	Starch	No	E	Arab et al., 2000
Thermococcus barophilus CNCMI 1946	37	75–95	85	4.5–9.5	7	1–4	3	Yeast extract, and peptone	No	E	Marteinsson et al., 1999
Thermococcus barossii DSMZ 9535	60	60–92	82.5	3–9	6.5/7.5	1–4	2	Tryptone, yeast extract, and malto-oligosaccharides			Duffaud et al., 1998
Thermococcus chtininophagus DSM 10152	46.5	60–93	85	3.5–9	6.7	0.8–8	2	Complex substrates, and chitin	No	E	Huber et al., 1996
Thermococcus siculi DSM 12349	55.8	50–93	85	5.0–9.0	7	0.1–0.4	0.2	Peptides	Yes	E	Grote et al., 1999
Thermococcus alcaliphilus DSM 10322	42.4	54–91	85	6.5–10.5	9.0	0.1–0.6	0.2–0.3	Peptides	Yes	E	Keller et al., 1995
Thermococcus waiotapuensis DSM 12768	50.4	60–90	85	5–8	7	Up to 13.9	5.4	Complex substrates, starch, maltose, and pyruvate	Yes	R	Gonzalez et al., 1999
Thermococcus fumiculans CIP 104690	54–55	73–103	90	4.5–9.5	8.5	0.6–4	1.3–2.6	Complex substrates, and pyruvate	Yes	E	Godfroy et al., 1996
Thermococcus sibiricus DSMZ 12597	38.4	40–88	78	5.8–9	7.5	0.5–7	1.8–2	Peptides	n.d.	E	Miroshnichenko et al., 2001
Thermococcus atlanticus CIP-107420T	49.8	70–95	85	4–9	7	1.5–4.6	30	Peptides	No	E	Cambon-Bonavita et al., 2003

Abbreviations: opt., optimal; n.d., no data available; E, elemental sulfur stimulates growth; and R, elemental sulfur is required for growth.
[a]Complex substrates: yeast extract, tryptone, peptone.

protein-dependent transport systems exhibit an unusually high affinity for the sugar, with a K_m in the submicromolar range. While glycolysis in thermophilic bacteria proceeds in a conventional way, glucose catabolism by Thermococcales (as well as in other hyperthermophilic archaea) differs from the canonical pathways, involves novel enzymes, and shows unique control. In an effort to understand the metabolism of cellobiose in *P. furiosus*, a binding protein-dependent ABC transport system for oligosaccharides was discovered. The 70-kDa protein is responsible for the uptake of cellobiose and most other α-glucosides (Koning et al., 2001).

Two major pathways are known to be involved in the degradation of glucose in prokaryotes: the Embden-Meyerhof (EM) and the Entner-Doudoroff (ED) pathways. In general, they differ in the key enzyme acting on glucose or glucose-6-phosphate and in the subsequent aldolytic cleavage of the intermediates fructose-1,6 biphosphate (EM) and 2-keto-3-deoxy-6-phosphogluconate (ED). So far, sugar degradation has been analyzed in representative species of the genera *Thermococcus* and *Pyrococcus*. The analysis included 1) determination of ^{13}C-labeling patterns by ^{1}H- and ^{13}C-NMR spectroscopy of fermentation products derived from pyruvate after fermentation of specifically ^{13}C-labeled glucose by cell suspensions, 2) identification of intermediates of sugar degradation after conversion of ^{14}C-labeled glucose by cell extracts, and 3) measurements of enzyme activities in cell extracts (Schönheit and Schäfer, 1995; De Vos et al., 1998). It has been established that in the three Thermococcales, *P. furiosus*, *T. celer* and *T. litoralis*, glycolysis appears to occur via a modified EM pathway. This pathway is unusual because the hexose kinase and phosphofructokinase steps are dependent on ADP rather than ATP, and a novel tungsten-containing enzyme termed "glyceraldehyde-3-phosphate:ferredoxin oxidoreductase" (GAPOR) replaces the expected glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and phosphoglycerate kinase. In contrast, other thermophilic Archaea (like *Sulfolobus solfataricus* and *Thermoplasma acidophilum*) degrade glucose via the ED pathway, and hyperthermophilic bacteria belonging to the order Thermotogales degrade glucose via conventional forms of the EM and ED pathways (Schönheit and Schäfer, 1995; De Vos et al., 1998). A final step in sugar fermentation is the conversion of acetyl-CoA into acetate that produces ATP. Also in this crucial step, the Thermococcales are unique because they have a single enzyme, an ADP-dependent acetyl-CoA synthase, while in bacteria this reaction is catalyzed by two different enzymes, phosphate acetyltransferase and acetate kinase (Fig. 2). In addition, *P. furiosus* has the capacity to convert pyruvate into alanine, which acts as an alternative electron sink. This reaction involves the combined activity of both alanine aminotransferase and glutamate dehydrogenase (Kengen et al., 1994).

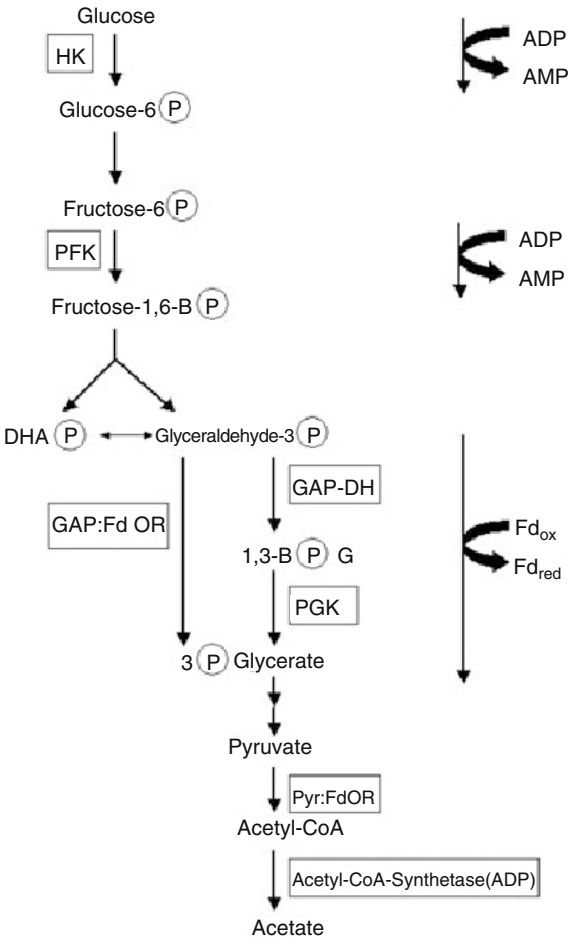

Fig. 2. Embden-Meyerhof-type glycolytic pathway in the genera *Pyrococcus* and *Thermococcus*. The phosphoryl-donor specificities (ADP-AMP) of hexokinase (HK) and 6-phosphofructokinase (PFK) and the enzyme proposed for glyceraldehyde-3-phosphate oxidation are indicated (GAP:FdOR, glyceraldehyde-3-phosphate:ferredoxin oxidoreductase; GAP-DH, glyceraldehyde-3-phosphate dehydrogenase; PGK, phosphoglycerate kinase; DHA-P, dihydroxyacetonephosphate; 1,3-BPG, 1,3-biphosphoglycerate; Fd*ox*, oxidized ferredoxin; Fd*red*, reduced ferredoxin; and Pyr:FdOR, pyruvate:ferredoxin oxidoreductase).

The operation of a new glycolytic pathway was demonstrated in nongrowing cells of *Thermococcus zilligii* by isotopic enrichment analysis of the end products derived from fermentation of ^{13}C-labeled glucose. The new pathway involves the formation of formate, derived from C-1 in glucose, via cleavage of a six-carbon carboxylic acid. The operation of a novel glycolytic strategy in *T. zilligii* with two branches diverging at the level of glucose-6-phosphate was demonstrated (Ron-

imus et al., 2001). Glucose is phosphorylated by an ADP-dependent hexokinase to glucose-6-phosphate, which is subsequently degraded by two glycolytic branches: an EM-type glycolytic pathway and a new route where formate is produced by a reaction involving cleavage of the C-1 carboxylic group of a six-carbon compound to yield formate and a pentose phosphate. By analogy with the pyruvate-formate-lyase reaction, it was suggested that the six-carbon compound is a β-ketoacid, such as 2-keto-3-deoxy-6-phosphogluconate, derived from 6-phosphogluconate. The contribution of the novel glycolytic branch was twice as high as that of the EM-type pathway when cells were grown on tryptone, and the inverse relationship was found for cells grown in the presence of glucose. This is the first report of a glycolytic pathway involving the formation of formate from C-1 in glucose. It is noteworthy that the most atypical member of the Thermococcales, *T. zilligii*, possesses also this unusual glycolytic feature (Ronimus et al., 1999; Ronimus et al., 2001).

The pathways of peptide metabolism have been well studied in *P. furiosus*. Amino acid catabolism in *P. furiosus* is thought to involve four distinct 2-keto acid oxidoreductases that convert transaminated amino acids into their corresponding coenzyme A (CoA) derivatives (Blamey and Adams, 1993; Mai and Adams, 1994; Mai and Adams, 1996; Heider et al., 1996). These CoA derivatives, together with acetyl-CoA produced from glycolysis via pyruvate, are then transformed to their corresponding organic acids by two acetyl-CoA synthetases unique to archaea, with concomitant substrate-level phosphorylation to form ATP. Alternatively, it has been postulated that depending on the redox balance of the cell, 2-keto acids are decarboxylated to aldehydes and then oxidized to form carboxylic acids by a second tungsten-containing enzyme, aldehyde:ferredoxin oxidoreductase (AOR; Mai and Adams, 1996). A third enzyme of this type, termed "formaldehyde:ferredoxin oxidoreductase" (FOR), is thought to be involved in the catabolism of basic amino acids (Roy et al., 1999; Adams et al., 2001).

Molecular Biology

The DNA G+C content of members of Thermococcales varies from 37.5 mol% for *Pyrococcus woesei* to 60 mol% for *Thermococcus barossii*. The abyssal strains *P. abyssi*, *P. horiskoshii* and *P. glycovorans* are characterized by a higher G+C (44–47 mol%) content than that of the coastal strains *P. furiosus* and *P. woesei* (38 mol%). A G+C content higher than 40 mol% is a typical feature of most *Thermococcus* species, but also in this genus, a distinction can be made. According to Godfroy et al. (1997), the strains of the genus *Thermococcus* can be divided on the basis of their G+C contents into the following two groups: 1) a group of strains with high G+C content (50–58 mol%), including the strains from shallow marine environment, *T. celer* and *T. stetteri,* and three deep-sea species *T. profundus*, *T. peptinophilus* and *T. funiculans*; and 2) a group of strains with low G+C content (38–47 mol%), including deep-sea strains of *T. chitinophagus*, *T. alcaliphilus* and *T. barophilus*, an organism from a shallow marine environment, *T. litoralis*, and the microorganism from a terrestrial high temperature oil reservoir, *Thermococcus sibiricus* (Table 1).

Phylogenetic studies based on 16S rDNA analysis reveal that *Pyrococcus* and *Palaeococcus* strains are clustered separately from *Thermococcus* species. However, the topology of the dendogram based on neighbor-joining algorithms clusters the Thermococcus species in a different number of branches, thus indicating that at the phylogenetic level, diversity in the same genus within high temperature environments is remarkable (Fig. 3).

The recent advances in genome projects have provided a considerable amount of data, which enables genomes of distant organisms to be compared in a comprehensive and integrative way. Comparisons of closely related species constitute a complementary approach crucial to the understanding of genome evolution. At the genomic level, these comparisons provide a unique opportunity to understand the mechanisms that determine chromosomal organization and evolution. At the proteomic level, this powerful strategy can be used to assess the genuine extent of gene losses and gains that lead to the observed divergence of coding capacity. Comparison of the genomes of Thermococcales has been already performed on three closely related species: *Pyrococcus abyssi* (Cohen et al., 2003; Genoscopewebsite http:\\www.genoscope.cns.fr), *Pyrococcus horikoshii* (Kawarabayasi et al., 1998; Kawarabayasi et al., 2001; The National Institute of Technology and Evaluation(NITE) website http:\\www.bio.nite.go.jp) and *Pyrococcus furiosus* (Maeder et al., 1999; Lecompte et al., 2001; Environmental Genome Project sponsored by the National Institute of Environmental Health Sciences http:\\www.genome.utah.edu; ORNL website http:\\www.ornl.gov). Several genome features of *P. abyssi*, *P. horikoshii* and *P. furiosus* affirm the close relationship among the three species, including similar G+C content and RNA elements, like rRNA and tRNA. At the genomic level, the comparison reveals that a differential conservation among four regions of the *Pyrococcus* chromosomes correlates with the location of genetic elements mediating DNA reorganization. At the proteomic level, the closer proximity of

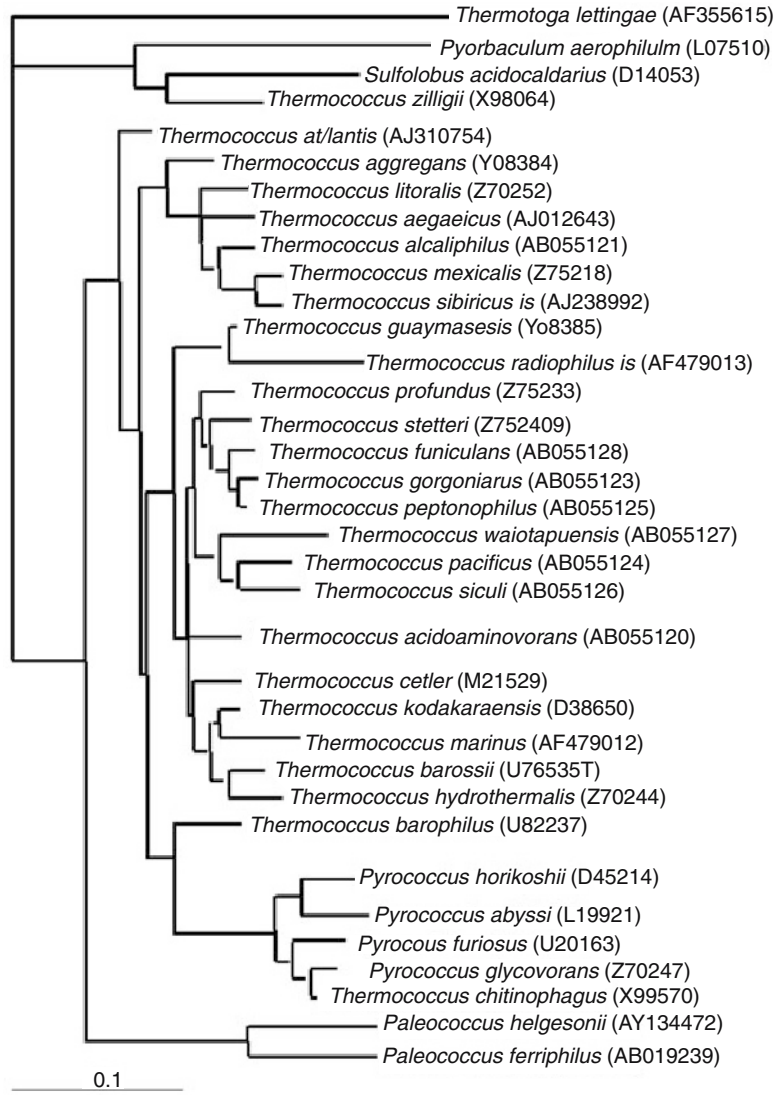

Fig. 3. Phylogenetic tree of the order Thermococcales as derived from neighbor-joining analysis of 16S rRNA. The tree was constructed by maximum-likelihood analysis using the program CLUSTAL W (Higgins et al., 1996). The 16S rRNA gene sequences were all obtained from GenBank. The accession number of the sequences is indicated within the brackets. Bar indicates one substitution per 100 nucleotides.

P. abyssi and *P. horikoshii* is affirmed by their average amino acid identity (77%) and their chromosomal organization. Nevertheless, the evolutionary distance between *P. abyssi* and *P. horikoshii* is not negligible relative to *P. furiosus* because the average amino acid identities are also high between *P. furiosus* and the two other species (72% with *P. abyssi* and 73% with *P. horikoshii*). The comparison of the three *Pyrococcus* species sheds light on specific selection pressure acting both on their coding capacities and on their evolutionary rates. The two independent methods, the "reciprocal best hits" approach and a new distance-ratio analysis, allow detection of the false orthology relationships within the *Pyrococcus* lineage. Such analyses reveal a high amount of differential gains and losses of genes since the three closely related species diverged. The resulting polymorphism is probably linked to an adaptation of these free-living organisms to differential environmental constraints.

Enzymology

Because members of Thermococcales grow at high temperature, their enzymes are highly thermoactive and thermostable. A large number of enzymes show no significant loss of activity after several hours at 100°C and are even active at temperatures that exceed the optimal growth temperature of the organism from which they were isolated (Bertoldo and Antranikian, 2002). These properties make hyperthermophiles very attractive for new biotechnological applications. To date, a large number of extracellular and intracellular enzymes have been characterized. They include the extracellular enzymes like amylases, pullulanases, α-glucosidases and proteases but also intracellular enzymes like dehydrogenases, oxidoreductases and DNA polymerases (Table 2). Interestingly, the ability of hyperthermophiles to produce cellulases, xylanases and pectinases seems to be very limited,

Table 2. Properties of enzymes from Thermococcales with potential biotechnological implications.

Enzymes	Organism[a]	Enzyme properties			Remarks
		Optimal temperature	Optimal pH	Mw (kDa)	
α-Amylase	*Pyrococcus furiosus* (100)	100	6.5–7.5	129	Purified/cloned/intracellular
		100	7.0	68	Purified/cloned/extracellular
	Pyrococcus kodakaraensis	90	6.5	49.5	Purified/cloned/extracellular
	Pyrococcus woesi (100)	100	5.5	68	Purified/extracellular
	Thermococcus celer (85)	90	5.5	—	Crude extract
	Thermococcus profundus DT5432 (80)	80	5.5	42	Purified/cloned/"Amy S"
	Thermococcus profundus (80)	80	4.0–5.0	42	Purified/"Amy L"
	Thermococcus aggregans (85)	95	6.5	—	Cloned
Pullulanase type II	*Pyrococcus woesei* (100)	100	6.0	90	Purified/cloned/cell associated
	Thermococcus celer (85)	90	5.5	—	Crude extract
	Thermococcus litoralis (90)	98	5.5	119	Purified/extracell./glycoprotein
	Thermococcus hydrothermalis (80)	95	5.5	128	Purified/extracell./glycoprotein
Pullulan-hydrolase type III	*Thermococcus aggregans* (85)	100	6.5	83	Purified/cloned
CGTase	*Thermococcus* sp. (75)	100	2.0	83	Purified
α-Glucosidase	*Thermococcus strain* AN1 (80)	130	—	63	Purified/extracell./glycoprotein
	Thermococcus hydrothermalis (80)	—	—	—	Cloned
	Pyrococcus furiosus (100)	—	5.0–6.0	125	Pur./extracellular
	Pyrococcus woesei (100)	100	5.0–5.5	90	Pur./cloned/intracellular
	Thermococcus chitinophagus (80)	70	7.0	70	Purified
Chitinase	*Pyrococcus kodakaraensis* (95)	85	5.0	135	Purified/cloned
	Thermococcus chitinophagus (80)	70	7.0	70	Purified
Exoglucanase	*Pyrococcus furiosus* (100)	100	6.0	35.9	Cloned
β-Glycosidase	*Pyrococcus furiosus* (100)	102–105	—	230/58	Purified/cloned
Serine protease	*Pyrococcus furiosus* (100)	85	6.3	124/19	Protease I/purified
		—	—	105/80	Pyrolysin/pur./cloned
	Thermococcus aggregans (75)	90	7.0	—	Crude extract
	Thermococcus celer (85)	95	7.5	—	Crude extract
	Thermococcus litoralis (90)	95	9.5	—	Crude extract
	Thermococcus stetteri (75)	85	8.5	68	Pur./cloned
Thiol protease	*Pyrococcus kodakaraensis* (95)	110	7	44	Purified
DNA ligase (*Pfu*)	*Pyrococcus furiosus* (100)	90	6.0	35.9	Cloned
DNA polymerase	*Thermococcus litoralis* (90)	70–80	8.8	98.9	Cloned/purified
DNA polymerase	*Thermococcus* strain 9°N-7 (90)	70–80	—	89.6	Cloned/purified
DNA polymerase	*Thermococcus aggregans* (90)	80	7.5	90	Cloned/purified
DNA polymerase	*Pyrococcus furiosus* (100)	72–78	8.0–9.0	90.1	Cloned/Purified
DNA polymerase	*Pyrococcus kodakaraensis* (95)	75	7.5	—	Purified

Symbol: –, not determined.

[a]Values in parentheses give the optimal growth temperature for each organism in °C.

and only in few cases the formation of cellobiohydrolase has been reported. Among the intracellular enzymes, the DNA polymerase and DNA ligase from *Pyrococcus* sp. (Southworth et al., 1996) and the DNA polymerase from *Thermococcus* strains especially have attracted further interest because of their potential in commercial applications to PCR. For reviews on the enzymology of hyperthermophilic archaea and their potential applications refer to the following articles (Sunna et al., 1997; Niehaus et al., 1999; Lévêque et al., 2000; Bertoldo and Antranikian, 2002; Bohlke et al., 2002).

Literature Cited

Achenbach-Richter, L., R. Gupta, W. Zillig, and C. R. Woese. 1988. Rooting the archaebacterial tree: The pivotal role of Thermococcus celer in archaebacterial evolution. Syst. Appl. Microbiol. 10:231–240.

Adams, M. W., J. F. Holden, A. L. Menon, G. J. Schut, A. M. Grunden, C. Hou, A. M. Hutchins, F. E. Jenney Jr., C. Kim, K. Ma, G. Pan, R. Roy, R. Sapra, S. V. Story, and M. F. Verhagen. 2001. Key role for sulfur in peptide metabolism and in regulation of three hydrogenases in the hyperthermophilic archaeon Pyrococcus furiosus. J. Bacteriol. 183(2):716–724.

Arab, H., H. Volker, and M. Thomm. 2000. Thermococcus aegaeicus sp. nov. and Staphylothermus hellenicus sp. nov., two novel hyperthermophilic archaea isolated from geothermally heated vents off Palaeochori Bay, Milos, Greece. Int. J. Syst. Evol. Microbiol. 50 Pt 6:2101–2108.

Balch, W. E., G. E. Fox, L. J. Magrum, C. R. Woese, and R. S. Wolfe. 1979. Methanogens: Reevaluation of a unique biological group. Microbiol. Rev. 43(2):260–296.

Barbier, G., A. Godfroy, J. R. Meunier, J. Querellou, M. A. Cambon, F. Lesongeur, P. A. Grimont, and G. Raguenes. 1999. Pyrococcus glycovorans sp. nov., a hyperthermophilic archaeon isolated from the East Pacific Rise. Int. J. Syst. Bacteriol. 49 Pt 4:1829–1837.

Bertoldo, C., and G. Antranikian. 2002. Starch-hydrolyzing enzymes from thermophilic archaea and bacteria. Curr. Opin. Chem Biol. 2:151–160.

Biller, K. F., I. Kato, and H. Markl. 2002. Effect of glucose, maltose, soluble starch, and CO_2 on the growth of the hyperthermophilic archaeon Pyrococcus furiosus. Extremophiles 6(2):161–166.

Blamey, J. M., and M. W. Adams. 1993. Purification and characterization of pyruvate ferredoxin oxidoreductase from the hyperthermophilic archaeon Pyrococcus furiosus. Biochim. Biophys. Acta 1161(1):19–27.

Blamey, J., M. Chiong, C. Lopez, and E. Smith. 1999. Optimization of the growth conditions of the extremely thermophilic microorganisms Thermococcus celer and Pyrococcus woesei. J. Microbiol. Meth. 38(1–2):169–175.

Blumentals, I. I., S. H. Brown, R. N. Schicho, A. K. Skaja, H. R. Costantino, and R. M. Kelly. 1990. The hyperthermophilic archaebacterium, Pyrococcus furiosus. Development of culturing protocols, perspectives on scaleup, and potential applications. Ann. NY Acad. Sci. 589:301–314.

Bohlke, K., F. M. Pisani, M. Rossi, and G. Antranikian. 2002. Archaeal DNA replication: Spotlight on a rapidly moving field. Extremophiles 6(1):1–14.

Britton, K. L., P. J. Baker, K. M. Borges, P. C. Engel, A. Pasquo, D. W. Rice, F. T. Robb, R. Scandurra, T. J. Stillman, and K. S. Yip. 1995. Insights into thermal stability from a comparison of the glutamate dehydrogenases from Pyrococcus furiosus and Thermococcus litoralis. Eur. J. Biochem. 229(3):688–695.

Cambon-Bonavita, M. A., F. Lesongeur, P. Pignet, N. Wery, C. Lambert, A. Godfroy, J. Querellou, and G. Barbier. 2003. Extremophiles, thermophily section, species description Thermococcus atlanticus sp. nov., a hyperthermophilic Archaeon isolated from a deep-sea hydrothermal vent in the Mid-Atlantic Ridge. Extremophiles 7(2):101–109.

Canganella, F., J. M. Gonzalez, M. Yanagibayashi, C. Kato, and K. Horikoshi. 1997a. Pressure and temperature effects on growth and viability of the hyperthermophilic archaeon Thermococcus peptonophilus. Arch. Microbiol. 168(1):1–7.

Canganella, F., W. J. Jones, A. Gambacorta, and G. Antranikian. 1997b. Biochemical and phylogenetic characterization of two novel deep-sea Thermococcus isolates with potentially biotechnological applications. Arch. Microbiol. 167(4):233–238.

Canganella, F., W. J. Jones, A. Gambacorta, and G. Antranikian. 1998. Thermococcus guaymasensis sp. nov. and Thermococcus aggregans sp. nov., two novel thermophilic archaea isolated from the Guaymas Basin hydrothermal vent site. Int. J. Syst. Bacteriol. 48 Pt 4:1181–1185.

Cohen, G. N., V. Barbe, D. Flament, M. Galperin, R. Heilig, O. Lecompte, O. Poch, D. Prieur, J. Querellou, R. Ripp, J. C. Thierry, J. Van der Oost, J. Weissenbach, Y. Zivanovic, and P. Forterre. 2003. An integrated analysis of the genome of the hyperthermophilic archaeon Pyrococcus abyssi. Molec. Microbiol. 47(6):1495–1512.

Costantino, H. R., S. H. Brown, and R. M. Kelly. 1990. Purification and characterization of an alpha-glucosidase from a hyperthermophilic archaebacterium, Pyrococcus furiosus, exhibiting a temperature optimum of 105 to 115 degrees C. J. Bacteriol. 172(7):3654–3660.

De Vos, W. M., S. W. Kengen, W. G. Voorhorst, and J. Van der Oost. 1998. Sugar utilization and its control in hyperthermophiles. Extremophiles 2:201–205.

Diederichs, K., J. Diez, G. Greller, C. Muller, J. Breed, C. Schnell, C. Vonrhein, W. Boos, and W. Welte. 2000. Crystal structure of MalK, the ATPase subunit of the trehalose/maltose ABC transporter of the archaeon Thermococcus litoralis. EMBO J. 19(22):5951–5961.

Dirmeier, R., M. Keller, D. Hafenbradl, F. J. Braun, R. Rachel, S. Burggraf, and K. O. Stetter. 1998. Thermococcus acidaminovorans sp. nov., a new hyperthermophilic alkalophilic archaeon growing on amino acids. Extremophiles 2(2):109–114.

Duffaud, G. D., O. B. d'Hennezel, A. S. Peek, A. L. Reysenbach, and R. M. Kelly. 1998. Isolation and characterization of Thermococcus barossii, sp. nov., a hyperthermophilic archaeon isolated from a hydrothermal vent flange formation. Syst. Appl. Microbiol. 21(1):40–49.

Erauso, G., Reysenbach, A. L., Godfroy, A., Meunier, J. R., Crump, B., Partensky, F., Baross, J. A., Marteinsson, V., Barbier, G., Pace, N. R., and Prieur, D. 1993. Pyrococcus abyssi sp. nov., a new hyperthermophilic archaeon iso-

lated from a deep-sea hydrothermalvent. Arch. Microbiol. 160:338–349.

Fiala, G., and K. O. Stetter. 1986. Pyrococcus furiosus sp. nov. represents a novel genus of marine heterotrophic archaebacteria growing optimally at 100°C. Arch. Microbiol. 145:56–61.

Godfroy, A., J. R. Meunier, J. Guezennec, F. Lesongeur, G. Raguénès, A. Rimbault, and G. Barbier. 1996. Thermococcus fumicolans sp. nov., a new hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent in the north Fiji Basin. Int. J. Syst. Evol. Microbiol. 46(4):1113–1119.

Godfroy, A., F. Lesongeur, G. Raguenes, J. Querellou, E. Antoine, J. R. Meunier, J. Guezennec, and G. Barbier. 1997. Thermococcus hydrothermalis sp. nov., a new hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent. Int. J. Syst. Bacteriol. 47:622–626.

Gonzalez, J. M., C. Kato, and K. Horikoshi. 1995. Thermococcus peptonophilus sp. nov., a fast-growing, extremely thermophilic archaebacterium isolated from deep-sea hydrothermal vents. Arch. Microbiol. 164(3):159–164.

Gonzalez, J. M., Y. Masuchi, F. T. Robb, J. W. Ammerman, D. L. Maeder, M. Yanagibayashi, J. Tamaoka, and C. Kato. 1998. Pyrococcus horikoshii sp. nov., a hyperthermophilic archaeon isolated from a hydrothermal vent at the Okinawa Trough. Extremophiles 2(2):123–130.

Gonzalez, J. M., D. Sheckells, M. Viebahn, D. Krupatkina, K. M. Borges, and F. T. Robb. 1999. Thermococcus waiotapuensis sp. nov., an extremely thermophilic archaeon isolated from a freshwater hot spring. Arch. Microbiol. 172(2):95–101.

Greller, G., R. Horlacher, J. DiRuggiero, and W. Boos. 1999. Molecular and biochemical analysis of MalK, the ATP-hydrolyzing subunit of the trehalose/maltose transport system of the hyperthermophilic archaeon Thermococcus litoralis. J. Biol. Chem. 274(29):20259–20264.

Greller, G., R. Riek, and W. Boos. 2001. Purification and characterization of the heterologously expressed trehalose/maltose ABC transporter complex of the hyperthermophilic archaeon Thermococcus litoralis. Eur. J. Biochem. 268(14):4011–4018.

Grote, R., L. Li, J. Tamaoka, C. Kato, K. Horikoshi, and G. Antranikian. 1999. Thermococcus siculi sp. nov., a novel hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent at the Mid-Okinawa Trough. Extremophiles 3(1):55–62.

Gruyer, S., E. Legin, C. Bliard, S. Ball, and F. Duchiron. 2002. The endopolysaccharide metabolism of the hyperthermophilic archeon Thermococcus hydrothermalis: Polymer structure and biosynthesis. Curr. Microbiol. 44(3):206–211.

Heider, J., X. Mai, and M. W. Adams. 1996. Characterization of 2-ketoisovalerate ferredoxin oxidoreductase, a new and reversible coenzyme A-dependent enzyme involved in peptide fermentation by hyperthermophilic archaea. J. Bacteriol. 178(3):780–787.

Higgins, D. G., J. D. Thompson, and T. J. Gibson. 1996. Using CLUSTAL for multiple sequence alignments. Meth. Enzymol. 266:383–402.

Holden, J. F., and J. A. Baross. 1993. Enhanced thermotolerance and temperature-induced changes in protein composition in the hyperthermophilic archaeon ES4. J. Bacteriol. 175(10):2839–2843.

Horlacher, R., K. B. Xavier, H. Santos, J. DiRuggiero, M. Kossmann, and W. Boos. 1998. Archaeal binding protein-dependent ABC transporter: Molecular and biochemical analysis of the trehalose/maltose transport system of the hyperthermophilic archaeon Thermococcus litoralis. J. Bacteriol. 180(3):680–689.

Huber, R., J. Stöhr, S. Hohenhaus, R. Rachel, S. Burggraf, H. W. Jannasch, and K. O. Stetter. 1996. Thermococcus chitonophagus sp. nov., a novel, chitin-degrading, hyperthermophilic archaeum from a deep-sea hydrothermal vent environment. Arch. Microbiol. 64:255–264.

Kawarabayasi, Y., M. Sawada, H. Horikawa, Y. Haikawa, Y. Hino, S. Yamamoto, M. Sekine, S. Baba, H. Kosugi, A. Hosoyama, Y. Nagai, M. Sakai, K. Ogura, R. Otsuka, H. Nakazawa, M. Takamiya, Y. Ohfuku, T. Funahashi, T. Tanaka, Y. Kudoh, J. Yamazaki, N. Kushida, A. Oguchi, K. Aoki, and H. Kikuchi. 1998. Complete sequence and gene organization of the genome of a hyperthermophilic archaebacterium, Pyrococcus horikoshii OT3. DNA Res. 5(2):55–76.

Kawarabayasi, Y. 2001. Genome of Pyrococcus horikoshii OT3. Meth. Enzymol. 330:124–134.

Keller, M., F. J. Braun, R. Dirmeier, D. Hafenbradl, S. Burggraf, R. Rachel, and K. O. Stetter. 1995. Thermococcus alcaliphilus sp. nov., a new hyperthermophilic archaeum growing on polysulfide at alkaline pH. Arch. Microbiol. 164(6):390–395.

Kengen, S. W., E. J. Luesink, A. J. Stams, and A. J. Zehnder. 1993. Purification and characterization of an extremely thermostable beta-glucosidase from the hyperthermophilic archaeon Pyrococcus furiosus. Eur. J. Biochem. 213(1):305–312.

Kengen, S. W., F. A. de Bok, N. D. van Loo, C. Dijkema, A. J. Stams, and W. M. de Vos. 1994. Evidence for the operation of a novel Embden-Meyerhof pathway that involves ADP-dependent kinases during sugar fermentation by Pyrococcus furiosus. J. Biol. Chem. 269(26):17537–17541.

Kobayashi, T., Y. S. Kwak, T. Akiba, T. Kudo, and K. Horikoshi. 1994. Thermococccus profundus sp. nov., a new hyperthermophilic archaeon isolated from a deep-sea hydrothermal vent. Syst. Appl. Microbiol. 17:232–236.

Koning, S. M., M. G. Elferink, W. N. Konings, and A. J. Driessen. 2001. Cellobiose uptake in the hyperthermophilic archaeon Pyrococcus furiosus is mediated by an inducible, high-affinity ABC transporter. J. Bacteriol. 183(17):4979–4984.

Krahe, M., G. Antranikian, and H. Märkl. 1996. Fermentation of extremophilic microorganisms. FEMS Microbiol. Rev. 18:271–285.

Kwak, Y. S., T. Kobayashi, T. Akiba, K. Horikoshi, and Y. B. Kim. 1995. A hyperthermophilic sulfur-reducing archaebacterium, Thermococcus sp. DT1331, isolated from a deep-sea hydrothermal vent. Biosci. Biotechnol. Biochem. 59(9):1666–1669.

Lattuati, A., J. Guezennec, P. Metzger, and C. Largeau. 1998. Lipids of Thermococcus hydrothermalis, an archaea isolated from a deep-sea hydrothermal vent. Lipids 33(3):319–326.

Lecompte, O., R. Ripp, V. Puzos-Barbe, S. Duprat, R. Heilig, J. Dietrich, J. C. Thierry, and O. Poch. 2001. Genome evolution at the genus level: Comparison of three complete genomes of hyperthermophilic archaea. Genome Res. 11(6):981–993.

Lévêque, E., S. Janeek, and H. B. Belarbi. 2000. Thermophilic archaeal amylolytic enzymes. Enz. Microb. Technol. 1:3–14.

Maeder, D. L., R. B. Weiss, D. M. Dunn, J. L. Cherry, J. M. Gonzalez, J. DiRuggiero, and F. T. Robb. 1999. Divergence of the hyperthermophilic archaea Pyrococcus furiosus and P. horikoshii inferred from complete genomic sequences. Genetics 152(4):1299–1305.

Mai, X., and M. W. Adams. 1994. Indolepyruvate ferredoxin oxidoreductase from the hyperthermophilic archaeon Pyrococcus furiosus. A new enzyme involved in peptide fermentation. J. Biol. Chem. 269(24):16726–16732.

Mai, X., and M. W. Adams. 1996. Purification and characterization of two reversible and ADP-dependent acetyl coenzyme A synthetases from the hyperthermophilic archaeon Pyrococcus furiosus. J. Bacteriol. 178(20): 5897–5903.

Marteinsson, V. T., J. L. Birrien, A. L. Reysenbach, M. Vernet, D. Marie, A. Gambacorta, P. Messner, U. B. Sleytr, and D. Prieur. 1999. Thermococcus barophilus sp. nov., a new barophilic and hyperthermophilic archaeon isolated under high hydrostatic pressure from a deep-sea hydrothermal vent. Int. J. Syst. Bacteriol. 49 Pt 2:351–359.

Miroshnichenko, M. L., E. A. Bonch-Osmolovskaya, A. Neuner, N. A. Kostrikina, N. A. Chernych, and V. A. Alekseev. 1989. Thermococcus stetteri sp. nov., a new extremely thermophilic marine sulfur-metabolizing archaebacterium. Syst. Appl. Microbiol. 12:257–262.

Miroshnichenko, M. L., G. M. Gongadze, F. A. Rainey, A. S. Kostyukova, A. M. Lysenko, N. A. Chernyh, and E. A. Bonch-Osmolovskaya. 1998. Thermococcus gorgonarius sp. nov. and Thermococcus pacificus sp. nov.: Heterotrophic extremely thermophilic archaea from New Zealand submarine hot vents. Int. J. Syst. Bacteriol. 48 Pt 1:23–29.

Miroshnichenko, M. L., H. Hippe, E. Stackebrandt, N. A. Kostrikina, N. A. Chernyh, C. Jeanthon, T. N. Nazina, S. S. Belyaev, and E. A. Bonch-Osmolovskaya. 2001. Isolation and characterization of Thermococcus sibiricus sp. nov. from a Western Siberia high-temperature oil reservoir. Extremophiles 5(2):85–91.

Morikawa, M., Y. Izawa, N. Rashid, T. Hoaki, and T. Imanaka. 1994. Purification and characterization of a thermostable thiol protease from a newly isolated hyperthermophilic Pyrococcus sp. Appl. Environ. Microbiol. 60(12):4559–4566.

Neuner, A., H. W. Jannasch, S. Belkinn, and K. O. Stetter. 1990. Thermococcus litoralis sp. nov.: S new species of extremely thermophilic marine archaebacterium. Arch. Microbiol. 153:205–207.

Niehaus, F., C. Bertoldo, M. Kahler, and G. Antranikian. 1999. Extremophiles as a source of novel enzymes for industrial application. Appl. Microbiol. Biotechnol. 51(6):711–729.

Ronimus, R. S., A. Reysenbach, D. R. Musgrave, and H. W. Morgan. 1997. The phylogenetic position of the Thermococcus isolate AN1 based on 16S rRNA gene sequence analysis: A proposal that AN1 represents a new species, Thermococcus zilligii sp. nov. Arch. Microbiol. 168(3):245–258.

Ronimus, R. S., J. Koning, and H. W. Morgan. 1999. Purification and characterization of an ADP-dependent phosphofructokinase from Thermococcus zilligii. Extremophiles 3(2):121–129.

Ronimus, R. S., E. de Heus, and H. W. Morgan. 2001. Sequencing, expression, characterisation and phylogeny of the ADP-dependent phosphofructokinase from the hyperthermophilic, euryarchaeal Thermococcus zilligii. Biochim. Biophys. Acta 1517(3):384–391.

Roy, R., S. Mukund, G. J. Schut, D. M. Dunn, R. Weiss, and M. W. Adams. 1999. Purification and molecular characterization of the tungsten-containing formaldehyde ferredoxin oxidoreductase from the hyperthermophilic archaeon Pyrococcus furiosus: The third of a putative five-member tungstoenzyme family. J. Bacteriol. 181(4):1171–1180.

Schönheit, P., and T. Schäfer. 1995. Metabolism of hyperthermophiles. World J. Microbiol. Biotechnol. 11:26–57.

Selig, M., K. B. Xavier, H. Santos, and P. Schonheit. 1997. Comparative analysis of Embden-Meyerhof and Entner-Doudoroff glycolytic pathways in hyperthermophilic archaea and the bacterium Thermotoga. Arch. Microbiol. 167(4):217–232.

Southworth, M. W., H. Kong, R. B. Kucera, J. Ware, H. W. Jannasch, and P. F. B. 1996. Cloning of thermostable DNA polymerases from hyperthermophilic marine Archaea with emphasis on Thermococcus sp. 9°N-7 and mutations affecting 3'-5' exonuclease activity. PNAS 93:5281–5285.

Stetter, K. O. 1996. Hyperthermophiles in the history of life. CIBA Found. Symp. 202:1–10; discussion 11–8.

Sunna, A., M. Moracci, M. Rossi, and G. Antranikian. 1997. Glycosyl hydrolases from hyperthermophiles. Extremophiles 1(1):2–13.

Takahata, Y., T. Hoaki, and T. Maruyama. 2001. Starvation survivability of Thermococcus strains isolated from Japanese oil reservoirs. Arch. Microbiol. 176(4):264–270.

Takai, K., A. Sugai, T. Itoh, and K. Horikoshi. 2000. Palaeococcus ferrophilus gen. nov., sp. nov., a barophilic, hyperthermophilic archaeon from a deep-sea hydrothermal vent chimney. Int. J. Syst. Evol. Microbiol. 50 Pt 2:489–500.

Verhagen, M. F., A. L. Menon, G. J. Schut, and M. W. Adams. 2001. Pyrococcus furiosus: Large-scale cultivation and enzyme purification. Meth. Enzymol. 330:25–30.

Xavier, K. B., R. Peist, M. Kossmann, W. Boos, and H. Santos. 1999. Maltose metabolism in the hyperthermophilic archaeon Thermococcus litoralis: Purification and characterization of key enzymes. J. Bacteriol. 181(11):3358–3367.

Zillig, W., I. Holtz, D. Janecovic, W. Schäfer, and W. D. Reiter. 1983. The archaebacterium Thermococcus celer represents a novel genus within the thermophilic branch of the archaebacteria. Syst. Appl. Microbiol. 4:88–94.

Zillig, W., I. Holz, H. P. Klenk, J. Trent, S. Wunderl, D. Janekovic, E. Imsel, and B. Haas. 1987. Pyrococcus woesei, sp. nov., an ultra-thermophilic marine Archaebacterium, representing a novel order, Thermococcales. Syst. Appl. Microbiol. 9:62–70.