FEBS Letters 452 (1999) 22–25 FEBS 22120

Minireview

Extremophiles and their adaptation to hot environments

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Received 4 May 1999

Abstract Water-containing terrestrial, subterranean and submarine high temperature areas harbor a variety of hyperthermophilic bacteria and archaea which are able to grow optimally above 80°C. Hyperthermophiles are adapted to hot environments by their physiological and nutritional requirements. As a consequence, cell components like proteins, nucleic acids and membranes have to be stable and even function best at temperatures around 100°C. The chemolithoautotrophic archaeon *Pyrolobus fumarii* is able to grow at 113°C and, therefore, represents the upper temperature border of life. For the first time, (vegetative) cultures of *Pyrolobus* and *Pyrodictium* are able to survive autoclaving.

Key words: Extremophile; Hyperthermophile; Archae; Volcanism; Geothermal

1. Introduction

In an anthrophocentric view, environments hostile to man were designated as extreme. Some of them had originally been considered to be too extreme to support microbial life at all, like hot springs, cold arctic water, acidic and alkaline water, saturated salt brines and pressurized abyssal waters. By improvement of the culture conditions, however, 'extremophilic' microorganisms could be found thriving within such environments which are designated as 'extremophiles'. Depending on their optimal growth conditions, they are named thermophiles, psychrophiles, acidophiles, alcalophiles, halophiles and barophiles, respectively. At present, extremophilic microorganisms are exciting research objects, the same in basic as applied research.

The most extreme microorganisms so far were found within thermophiles. In contrast to usual thermophiles, hyperthermophiles grow fastest at temperatures between 80 and 106°C [1]. This is already the temperature range where some small biomolecules rapidly decompose [2,3]. The highest growth temperature observed at all is 113°C for *Pyrolobus fumarii* [4]. For species of the genera *Methanopyrus, Pyrodictium* and *Pyrolobus*, temperatures of 80°C and below are still too low to support growth [4–6]. In this paper, I will give an overview about hyperthermophiles, their taxonomic relationships, environmental adaptations and strategies of life.

2. Biotopes of hyperthermophiles

At present, hyperthermophiles are found in natural and artificial water-containing hot environments [6,7]. On land, volcanic exhalations from deep magma chambers heat up soils and surface waters, forming sulfur-harboring acidic solfataric fields and neutral to slightly alkaline hot springs [8]. The salinity of such terrestrial hydrothermal systems is usually low. Artificial biotopes are smouldering coal refuse piles and hot outflows from geothermal power plants [9,10]. Deep subterranean non-volcanic geothermally heated biotopes were discovered recently about 3500 meters below the bottom of the north sea and below the Alaskan north slope permafrost soil [11], where in situ temperatures are approximately 100°C. Marine biotopes may be shallow or abyssal hot sediments and hydrothermal systems. Deep sea hot 'smoker' chimneys consist of hydrothermally heated rock material which is teeming with a variety of hyperthermophiles (e.g. 10^8 cells of *Methanopyrus* spp. per g of rock [4,5,12,13]). A further submarine high temperature environment are active seamounts [14]. Marine hydrothermal systems generally contain the high salt concentration of sea water (about 3%) and exhibit a slightly acidic to alkaline pH (pH 5–8.5). Active volcanic environments usually harbor large amounts of steam, carbon dioxide, hydrogen sulfide, sulfur and variable quantities of carbon monoxide, hydrogen, methane, nitrogen and traces of ammonia. In addition, sulfate is present the same in solfataric fields as in sea water (sea water: about 30 mmol/l). Although unable to grow at the low ambient temperatures, hyperthermophiles are able to survive there at least for years.

3. Taxonomy and phylogenetic relation of hyperthermophiles

So far, about 70 species of hyperthermophilic bacteria and archaea are known. These have been isolated from different terrestrial and marine thermal areas [7,15,16]. Hyperthermophiles are very divergent, both in terms of their phylogeny and physiological properties, and are grouped into 29 genera in 10 orders. At present, small subunit ribosomal RNA (16S rRNA) sequence-based classification of prokaryotes is widely used for the recognition and characterization of novel taxonomic groups [17]. In addition, more traditional taxonomic features such as GC contents of DNA, DNA-DNA homology, morphology and physiological features (Table 1) may serve as separating characters in order to obtain a high resolution of taxonomy. The 16S rRNA-based phylogenetic tree exhibits three domains, the bacteria, archaea (the former 'archaebacteria') and eukarya [17,18]. Hyperthermophiles form a cluster around the root, occupying all the short deep phylogenetic branches within the archaeal and bacterial domains. As a rule, members of the deepest and shortest lineages exhibit

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PII: S0014-5793(99)00663-8

Table 1
Some basic features of hyperthermophiles (type species)

Species	Growth conditions						DNA	Morphology
	Temperature (°C)			pH	Aerobic (ae) or anaerobic (an)	Biotope (marine (m) or terrestrial (t)	G+C (mol %)	
	Minimum	Optimum	Maximum					
T. maritima	55	80	90	5.5–9	an	m	46	Rods with sheath
A. pyrophilus	67	85	95	5.4–7.5	ae	m	40	Rods
Sulfolobus acidocaldarius	60	75	85	1–5	ae	t	37	Lobed cocci
Metallosphaera sedula	50	75	80	1–4.5	ae	t	45	Cocci
Acidianus infernus	60	88	95	1.5–5	ae/an	t	31	Lobed cocci
Stygiolobus azoricus	57	80	89	1–5.5	an	t	38	Lobed cocci
Thermoproteus tenax	70	88	97	2.5–6	an	t	56	Regular rods
Pyrobaculum islandicum	74	100	103	5–7	an	t	46	Regular rods
Pyrobaculum aerophilum	75	100	104	5.8–9	ae/an	m	52	Regular rods
Thermofilum pendens	70	88	95	4–6.5	an	t	57	Slender regular rods
Desulfurococcus mobilis	70	85	95	4.5–7	an	m	51	Cocci
Thermosphaera aggregans	67	85	90	5–7	an	t	46	Cocci in aggregates
Sulfophobococcus zilligii	70	85	95	6.5–8.5	an	t	54	Cocci
Staphylothermus marinus	65	92	98	4.5–8.5	an	m	35	Cocci in aggregates
T. maritimus	75	88	98	5–7	an	m	49	Disks
Aeropyrum pernix	70	90	100	5–9	ae	m	67	Irregular cocci
Stetteria hydrogenophila	70	95	102	4.5–7	an	m	65	Irregular disks
Igneococcus islandicus	65	90	100	3.9–6.3	an	m	41	Irregular cocci
P. occultum	82	105	110	5–7	an	m	62	Disks with cannulae
Hyperthermus butylicus	80	101	108	7	an	m	56	Lobed cocci
P. fumarii	90	106	113	4.0–6.5	ae/an	m	53	Lobed cocci
Thermococcus celer	75	87	93	4–7	an	m	57	Cocci
Pyrococcus furiosus	70	100	105	5–9	an	m	38	Cocci
A. fulgidus	60	83	95	5.5–7.5	an	m	46	Irregular cocci
Ferroglobus placidus	65	85	95	6–8.5	an	m	43	Irregular cocci
Methanothermus sociabilis	65	88	97	5.5–7.5	an	t	33	Rods in clusters
Methanopyrus kandleri	84	98	110	5.5–7	an	m	60	Rods in chains
Methanococcus jannaschii	50	85	86	5.5–6.5	an	m	31	Irregular cocci
Methanococcus igneus	45	88	91	5–7.5	an	m	31	Irregular cocci

Table 2
Energy-yielding reactions in chemolithoautotrophic hyperthermophiles

Energy-yielding reaction	Genera
$2S^0+3O_2+2H_2O$ ® $2H_2SO_4$ ($2FeS_2+7O_2+2H_2O$ ® $2FeSO_4+2H_2SO_4$) = 'metal leaching'	*Sulfolobus*[a], *Acidianus*[a], *Metallosphaera*[a], *Aquifex*
$H_2+1/2O_2$ ® H_2O	*Aquifex, Acidianus*[a], *Metallosphaera*[a], *Pyrobaculum*[a], *Sulfolobus*[a]
H_2+HNO_3 ® HNO_2+H_2O	*Aquifex, Pyrobaculum*[a]
$4H_2+HNO_3$ ® NH_4OH+2H_2O	*Pyrolobus*
$H_2+6Fe(OH)_3$ ® $2Fe_3O_4+10H_2O$	*Pyrobaculum*[a]
$2FeCO_3+HNO_3+5H_2O$ ® $2Fe(OH)_3+HNO_2+2H_2CO_3$	*Ferroglobus*
$4H_2+H_2SO_4$ ® H_2S+4H_2O	*Archaeoglobus*[a]
H_2+S^0 ® H_2S	*Acidianus, Stygiolobus, Pyrobaculum*[a], *Thermoproteus*[a], *Pyrodictium*[a], *Igneococcus*
$4H_2+CO_2$ ® CH_4+2H_2O	*Methanopyrus, Methanothermus, Methanococcus*

[a]Facultatively heterotrophic.

the highest growth temperatures ('the shorter and deeper, the hotter' [19]). Within the bacteria, *Aquifex pyrophilus* and *Thermotoga maritima* exhibit the highest growth temperatures of 95 and 90°C, respectively (Table 1). Within the archaea, the organisms with the highest growth temperatures (between 102 and 113°C) are found within the *Crenarchaeota* (the former 'sulfur metabolizers') and the *Euryarchaeota* (the former 'methanogens halophiles') [7,17]. They are members of the crenarchaeal genera *Pyrolobus, Pyrodictium, Hyperthermus, Pyrobaculum, Igneococcus* and *Stetteria* and the euryarchaeal genera *Methanopyrus* and *Pyrococcus*.

In the future, based on total genome sequencing, deeper insights into the phylogenetic relationships of different gene groups will be available. At present, total genome sequences of the hyperthermophiles *T. maritima, Aquifex aeolicus* and *Archaeoglobus fulgidus* already revealed an unexpected gene exchange even between members of archaea and bacteria [20–23].

4. Strategies of life and environmental adaptations of hyperthermophiles

Hyperthermophiles are adapted to distinct environmental factors including the composition of minerals and gasses, pH, redox potential, salinity and temperature (Table 1).

Most of them exhibit a chemolithoautotrophic mode of nutrition: inorganic redox reactions serve as energy sources (chemolithotrophic) and CO_2 is the only carbon source required to build up organic cell material (autotrophic). Therefore, these organisms fix CO_2 by chemosynthesis and are designated chemolithoautotrophs. The energy-yielding reactions in chemolithoautotrophic hyperthermophiles are anaerobic and aerobic types of respiration (Table 2). H_2 serves as an important electron donor. Within the natural environment, it may be a component of volcanic gasses. Other electron donors are sulfide, sulfur and ferrous iron (Table 2). Like in mesophilic respiratory organisms, some hyperthermophiles may use oxygen as an electron acceptor. In contrast, however, oxygen-respiring hyperthermophiles are microaerophilic and therefore, grow only at reduced oxygen concentrations (even as low as 10 ppm: [4,24]). Anaerobic respiration types are the nitrate-, ferric iron-, sulfate-, sulfur- and carbon dioxide respirations (Table 2). Several chemolithoautotrophic hyperthermophiles are facultative heterotrophs (Table 2). Those are able to use organic material alternatively to inorganic nutrients whenever it is provided by the environment (e.g. by decaying cells). Heterotrophic hyperthermophiles gain energy either by aerobic or different types of anaerobic respiration, using organic material as electron donors, or by fermentation [25].

5. The basis of heat stability and the upper temperature limit for life

Due to the small size of cells of hyperthermophiles in the micrometer scale, any protection by insulation against the hot environment appears impossible. All cell components, therefore, have to be heat resistant. The molecular basis is unknown and still under investigation. Hyperthermophiles belong to two phylogenetically very different domains of life, the bacteria and archaea. Therefore, the strategies of molecular mechanisms including heat adaptation may be rather dissimilar depending on the phylogenetic position of the corresponding organism.

Cell components such as lipids, nucleic acids and proteins are usually quite heat-sensitive. Membrane lipids of the bacterial hyperthermophile *T. maritima* contain a novel glycerol ether lipid, 15,16-dimethyl-30-glyceryloxy-triacontanedioic acid. In contrast to the esterlipids known in mesophiles, it may significantly increase the stability of membranes against hydrolysis at high temperatures [26]. In contrast, membranes of all archaea (including even mesophiles) contain ether lipids derived from diphytanyl-glycerol or its dimer di(biphytanyl)-diglycerol, which exhibit a remarkable resistance against hydrolysis at high temperatures and an acidic pH [27]. Thermal resistance of the DNA double helix appears to be improved in hyperthermophiles by reverse gyrase, a unique type I DNA topoisomerase that causes positive supertwists for stabilization [28,29]. In addition, archaeal hyperthermophiles possess histones phylogenetically related to the eukaryotic core histones (e.g. H2A, H2B, H3 and H4). In vitro addition of histones to purified DNA increased its melting temperature drastically [30–32]. The secondary structure of ribonucleic acids appears to be stabilized against thermal destruction by an increased content of GC base pairs within the stem areas and by post-transcriptional modification [33–35]. Purified enzymes of hyperthermophiles usually show an extraordinary heat stability in vitro. For example, an amylase from *Pyrococcus woesei* is still active at 130°C [36]. Deeper insights into the stabilizing principles of hyperthermophilic proteins will most likely be obtained after comparison of three-dimensional structures with homologous mesophilic enzymes. At the upper temperature border of growth of hyperthermophiles, the function of heat-shock proteins appears to become essential. At 108°C, about 80% of the soluble protein of a crude extract of *Pyrodictium occultum* consisted of a heat inducible molecular

chaperone designated thermosome [37]. With the thermosome fully induced, cultures of *P. occultum*, the same as its specific relative *P. fumarii*, were able to survive 1 h autoclaving (121°C, 2 bar: [4,38]).

The upper temperature border of life is still unknown and depends on the stability of the biomolecules. At temperatures in the order of 100°C, already some low molecular weight compounds such as ATP and NAD hydrolyze quite rapidly (half life below 30 min in vitro) and thermolabile amino acids like cysteine and glutamic acid are decomposing [2,3]. The survival of organisms growing at these temperatures may be ensured by rapid re-synthesis of thermo-sensitive compounds. In addition, at higher temperatures, the proton permeability of membranes may become too high to maintain electrochemical proton gradients in order to gain energy (W. Konings, personal communication). When carefully sampled to avoid contamination by loose material from the smoker walls, the hot deep sea smoker fluids (200–350°C), in contrast to an earlier report, prove to be sterile (Jannasch and Stetter, personal communication). This finding is in line with the dramatic destruction of the building blocks of life at those temperatures and pressures [2,39,40]. The maximal growth temperature at which microbial life can exist could be found somewhere between 113 and 150°C. Within this temperature range, heat-sensitive biomolecules could possibly still be re-synthesized at biologically feasible rates. It is a great challenge for microbiologists to discover and cultivate such possibly existing exciting microbes.

Acknowledgements: The work presented from my laboratory was supported by grants of the Deutsche Forschungsgemeinschaft, the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie, the European Commission and the Fonds der Chemischen Industrie.

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