HYPERTHERMOPHILIC MICROORGANISMS

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Summary

Hyperthermophilic Archaea and Bacteria with optimal growth temperatures between 80 and 110° C have been isolated from geo- and hydrothermally heated terrestrial and submarine environments. Small subunit rRNA sequence comparisons indicate great phylogenetic diversity among the 32genera in 10 orders represented. Hyperthermophiles consist of anaerobic and aerobic chemolithoautotrophs and heterotrophs growing at neutral or acidic pH. Their outstanding heat resistance makes them as interesting objects for basic research as for biotechnology in the future.

1. Introduction

The first traces of life on Earth date back to the early Archaean age. Microfossils of cyanobacteria-like prokaryotes within fossil stromatolithes demonstrate the existence of life already 3.5 billion years ago. Possibly, life had already originated much earlier, possibly already at the end of the major period of meteorite impacts about 3.9 billion years ago. At that time, the Earth is generally assumed to had been much hotter than today. Questions arise about possible physiological properties, modes of energy aquisition, and kinds of carbon sources of the earliest organisms which may have made their living in a world of fire and water.

Today most life forms known are mesophiles adapted to ambient temperatures within a range from 15 to 45° C. Among bacteria, thermophiles (heat-lovers) have been recognized for some time which grow optimally (fastest) between 45 and 70° C. They thrive within sun-heated soils, self-heated waste dumps and thermal waters and are closely related to mesophiles. Since Louis Pasteur‘s time it had been generally assumed that vegetative (growing) cells of bacteria (including usual thermophiles) are quickly killed by temperatures of above 80° C. In contrast, during the last 25 years, hyperthermophilic bacteria and archaea (formerly the archaebacteria) with unprecedented properties have been isolated mostly from areas of volcanic activity. They grow between 80 and 113° C and represent the organisms at the currently accepted upper temperature border of life. Although these hyperthermophiles flourish above temperatures used in pasteurization, they are unable to propagate at 50° C or below. Organisms like *Pyrolobus* are so well adapted to heat that temperatures of 85° C are still too low to support growth. Hyperthermophiles belong to various phylogenetically distant groups and may represent rather ancient adaptations to their high temperature environments. In this chapter, I give an insight into the biotopes, modes of life, and phylogeny of hyperthermophiles, and show evidence for their primitiveness and their probable existence since the dawn of life in the early Archaean age.

2. Biotopes of hyperthermophiles

	Type of thermal area	
Characteristics	**Terrestrial**	**Marine**
Locations	Solfataric fields: steam-heated soils, mud holes, and surface waters; deeply originating hot springs; subterranean oil stratifications	Submarine hot springs, hot sediments and vents ("black smokers"); active seamounts.
Temperatures	Surface, up to 100 °C[a]; depth, above 100 °C	Up to ~400 °C ("black smokers")
Salinity	usually low (0.1–0.5% salt)	Usually ~3% salt
pH	0–10	5–8.5 (rarely: 3)
Major life-supporting gasses and nutrients	H_2O, CO_2, CO, CH_4, H_2, H_2S, S^0, $S_2O_3^{2-}$, SO_3^{2-}, SO_4^{2-}[b]), NH_4^+, N_2, NO_3^-, Fe^{2+}, Fe^{3+}, O_2 (surface)	

[a]At sea level
[b]Seawater contains ~30mmol L^{-1} of sulfate.

Table 1. Biotopes of hyperthermophiles

In nature, hyperthermophiles are found in water-containing volcanically and geothermally heated environments situated mainly along terrestrial and submarine tectonic fracture zones where plates are colliding (subduction) or moving away from each other (spreading). The temperatures in active volcanoes are much too high to support life (e.g. about 700°C to 1200°C in molten lava), but fumaroles and hot springs associated with volcanic activity provide much lower more suitable temperatures. Within saturated or superheated steam life is not possible and liquid water is a fundamental prerequisite. In several hyperthermophiles growth temperatures exceed 100°C, which is the boiling temperature of water at sea level. However, this is only possible under conditions increasing the boiling point of water (e.g. by elevated atmospheric, hydrostatic or osmotic pressure) in order to keep water in the liquid phase. For example, by an overpressure of one bar (200 kPa), the boiling point of water is raised from 100 to 120°C. This corresponds to a water depth of merely ten meters.

Due to the presence of reducing gasses (Table 1) and the low solubility of oxygen at high temperatures, biotopes of hyperthermophiles are essentially anaerobic. A great number of hyperthermophilic organisms exclusively depend on simple inorganic compounds provided by their environment to gain energy and to build up their cell components. Hyperthermophiles have been isolated from terrestrial and marine environments.

2.1. Terrestrial biotopes

Terrestrial biotopes of hyperthermophiles are mainly sulfur-containing solfataric fields, named after the Solfatara Crater at Pozzuoli (Naples area), Italy. Solfataric fields consist of soils, mud holes and surface waters heated by volcanic exhalations from magma chambers, a few kilometers below which are passing through porous rocks above. Very often, solfataric fields are situated at or in the close neighbourhood of active volcanoes and activity is greatly increased during eruption phases. For example, in 1980, one week before a fissure eruption, we had taken samples at the Krafla area in Iceland (Figure 1). A huge vigorously gassed mud crater had formed, throwing mud lumps tenths of meters high into the air.

Figure 1: Solfataric field at Krafla, Iceland

Solfataric fields exist in many countries all over the world, for example the Campi Flegrei in Pozzuoli, Italy; the Kerlingarfjöll mountains, Iceland; Yellowstone National Park, U.S.A.; Caldera Uzon, Kamchatka, Russia; Noboribetsu, Hokkaido, Japan; Tangkuban Prahu, Java, Indonesia and Rotorua, New Zealand. Depending on the altitude above sea level, the maximum water temperatures are up to 100°C (Table 1). However, there are lakes with hot springs at their bottom the temperatures of which exceed 100°C (e.g. Lake Yellowstone; Lake Tanganijka, Cape Banza). The salinity of solfataric fields is usually low. However, there are exceptions if they are situated at the beach (e.g. Faraglione, Vulcano, and Maronti beach, Ischia, both Italy).

The chemical composition of solfataric fields is very variable and depends on the site. Steam within the solfataric exhalations is mainly responsible for the heat transfer. CO_2 keeps the soils anaerobic and prevents penetration of oxygen into greater depths. In addition, H_2S reduces oxygen to water yielding elemental sulfur. An important gaseous energy source for hyperthermophiles is molecular hydrogen, which may be formed either pyrolytically from water or chemically from FeS and H_2S. Many solfataric fields are rich in iron minerals like ferric hydroxides, pyrite, and other ferrous sulfides. In soil profiles, two different zones are visible: an oxidized, strongly acidic (pH 0-3) upper layer of about 5 to 30 cm in thickness with an ochre colour caused by ferric hydroxides is overlaying a reduced zone below exhibiting a slightly acidic pH of between 4 and 6.5 and a bluish-black colour due to ferrous sulfides. Acidity within the surface layer is mainly based on the presence of sulfuric acid which may be formed either abiotically by oxidation of SO_2 or biotically (see below). Less usual compounds may be enriched at some sites, like magnetite or arsenic minerals auripigment and realgar in Geysirnaja Valley and Caldera Uzon, Kamchatka.

Sometimes, solfataric fields contain silicate-rich neutral to slightly alkaline (pH 7-10) hot springs originating from the depth. Their content of sulfur compounds is usually low. Under special conditions some of these hot springs may form geysirs, like Bunsen's geysir and Strokkur in Iceland, and those of Yellowstone Park, Rotorua, and Geysirnaja Valley. Possibly due to periodical exposure to atmospheric oxygen at high temperature, there is no evidence for larger amounts of hyperthermophiles within water of those "jumping springs". Geothermal areas of mainly neutral hot springs (pH 6.5-7.5; 60-103°C) with low sulfur contents are situated at Djibouti (Lac Abbé and Lac Assal), and at Lake Tanganijka, Kongo (Pemba and Cape Banza), at the African Rift Valley tectonic fracture system.

Active volcanoes may harbour hot crater lakes which are heated by fumaroles (e.g. Askja, Iceland and Karymsky, Kamchatka). Usually, those abound in sulfur and are very acidic and represent a further biotope of hyperthermophiles. Nothing is known about possible microbial life in the interior of active volcanoes. These mountains are assumed to be "sponges" which may contain a lot of hot water, possibly providing so far unexplored biotopes for hyperthermophiles.

First evidence for the presence of communities of hyperthermophiles within geothermally heated rocks 3500 meters below the surface had been demonstrated. Soils on the flanks of volcanoes, depending on the interior heat flow may harbour hyperthermophiles, too. For example, at the "Tramway Ridge" and southern crater on top of Mount Erebus, and on top of Mount Melbourne, both Antarctica, there are wet soils with temperatures between 60 and 65°C and pH 5-6 at an altitude of 3500 meters (Figure 2). They represent "islands" of thermophilic life within a deep-frozen continent.

Figure 2: Hot soils at top of Mount Melbourne, Antarctica (ice-free areas)

Very often solfataric fields and hot springs are situated in the neighbourhood or even within swamps, grassland or rainforests. Therefore, in addition to the inorganic nutrients initially present there, leaves, wood, insects etc. may fall in and may provide complex organic material as possible additional nutrients.

Artificial "solfataric fields" are smouldering coal refuse piles situated in humid areas like at Ronneburg, Thüringen, Germany. Usually, they contain elemental sulfur and exhibit an acidic pH. They harbour some hyperthermophiles similar to that of natural environments.

2.2. Marine biotopes

Marine biotopes of hyperthermophiles consist of various hydrothermal systems situated at shallow to abyssal depths. Similar to ambient sea water, submarine hydrothermal systems usually contain high concentrations of NaCl and sulfate and exhibit a slightly acidic to alkaline pH (5-8.5). Otherwise, the major gasses and life-supporting mineralic nutrients may be similar to that in terrestrial thermal areas (Table 1).

Shallow submarine hydrothermal systems are found in many parts of the world, mainly on beaches with active volcanism, like at Vulcano island, Ischia island and Stufe di Nerone, Naples (all: Italy); Ribeira Quente, Sao Miguel, Azores; Sangeang island, Indonesia; Obock, Gulf of Tadjura, Dijbouti; and Kunashiri, Japan. The hydrothermal system at Vulcano is situated close to the Porto di Levante harbour between the Fossa and Vulcanello volcanoes. It consists of H_2S-containing submarine fumaroles, hot springs and hot sandy sediments situated in a depth of one to ten meters with temperatures between 80 and 105°C (Figure 3). Various novel groups of marine hyperthermophiles had been discovered at this place, some members of which were found later to exist in other shallow and abyssal marine hydrothermal systems, too (see below; e.g. Table 3). A further recently discovered shallow submarine hydrothermal system is located at a depth of 105 meters at the Kolbeinsey Ridge (67°05'29"N; 18°42'53"W) which represents the northern continuation of the Mid Atlantic Ridge. It had been visited first by the German diving vessel *Geo.* Hot fluids with temperatures between 40 and 131°C and pH 6.5 are venting out from cracks and holes at the sea floor.

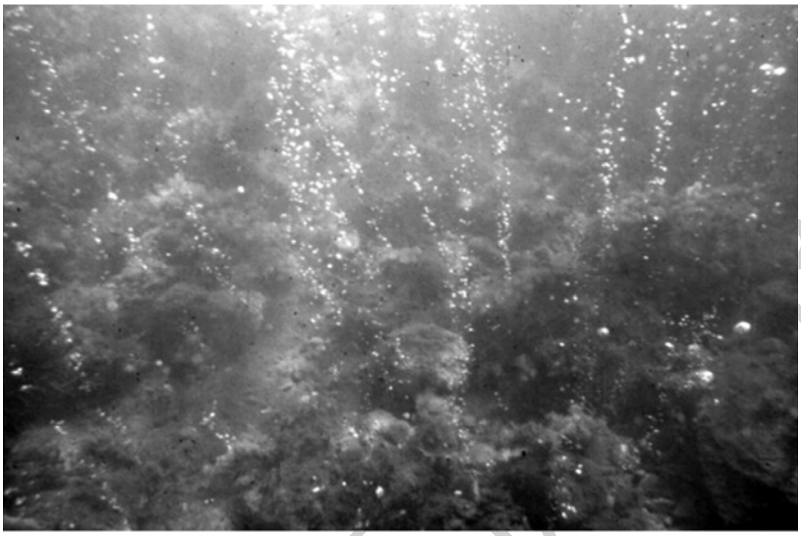

Figure 3: Outgassing at a shallow submarine hydrothermal system, Porto di Levante, Vulcano, Italy. Sea bottom about 6 meters deep

Most impressive are the deep sea "smoker" vents, where mineral-laden hydrothermal fluids with temperatures up to about 400° C escape into the cold (2.8° C) surrounding deep sea water and build up huge rock chimneys (Figure 4). Although these hot fluids are sterile, the surrounding porous smoker rock material appears to contain very steep temperature gradients which provide zones of suitable growth temperatures for hyperthermophiles. Some smoker rocks are teeming with hyperthermophiles (for example 10^8 cells of *Methanopyrus* per gram of rock inside a Mid Atlantic “Snake Pit“ hot vent chimney). Deep sea vents are located along submarine tectonic fracture zones and are known from several places which can be visited for sampling by deep dive submersibles like *Alvin* (U.S.A.), *Nautile* (France), and *Mir* (Russia). Powerful big black smoker systems do exist at the Mid Atlantic Ridge deep sea floor in a depth between 3000 and 4000 meters at the "TAG", "Snake Pit", "Broken Spurr" and the "Moose" sites. The temperature of the smoker fluids is usually between 200 and 360° C and the pH about 9. In addition to regular black smoker vent chimneys, beehive-shaped smoker vents are found among the Mid Atlantic Ridge from which fluids with temperatures of up to 244° C are seeping out. At the base of the Mid-Atlantic smokers there are usually hydrothermally heated lava rocks and minor sediments with much lower temperatures between 20 and 100° C.

Based on the gasses and mineral nutrients of the cooled-down venting fluids, an enormous chemosynthetic production of microbial biomass by thermophilic and mesophilic vent bacteria and archaea is going on, which represents the starting point at an (aphotic) food web which includes a tight assembly of various higher life forms like deep sea shrimps (e.g. Rimicaris excoculata), crabs, mussels and fishes. In contrast, the surrounding deep sea floor is low in nutrients and, therefore very poor in animals.

Further deep sea vents are situated in the Pacific, for example at 21° N at the East Pacific Rise in a depth of about 2700 meters (Figure 4). Many hot smokers with temperatures up to 400° C are found there, at a lava-covered deep sea floor without major sediments. Again, based on microbial biomass there is an "island" of very rich deep sea life including giant clams (*Calyptogena magnifica*), giant tube worms, up to 4 meters long (*Riftia pachyptila*) and many crabs and fishes.

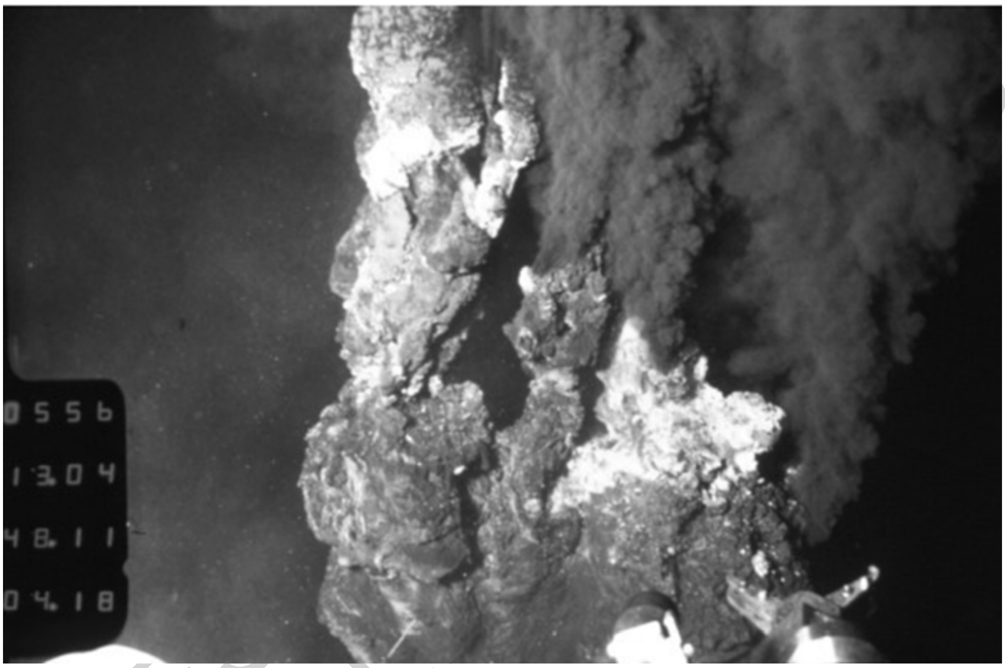

Figure 4: Abyssal hot "Black Smoker" chimneys at the East Pacific Rise 21°N. Depth: 2600 meters. Maximal fluid temperature: 365° C

Another extensive abyssal vent system is within the Guaymas Basin. It is located at the southern continuation of the San Andreas fault in a depth of 1500 meters between the Baja California peninsula and the main land of Mexico. The bottom of this ocean arm is covered with organic sediments up to 400 meters in thickness, which are heated hydrothermally. The temperature gradients within the sediments are steep. For example, we had measured at several sites temperatures of 85, 105, 132, and 150° C at a sediment depth of 5, 10, 15 and 35 cm, respectively. In addition to the heated sediments, assemblies of active black and white smoker chimneys and smoker flanches are present exhibiting fluid temperatures of between 308-326° C (pH 7). The heated flanch rocks, similar to walls of smoker vents, contain various species of hyperthermophiles.

	Members of genus (no. viable cells mL^{-1})			
Sample	***Pyrodictium***	***Pyrococcus***	***Thermococcus***	***Archaeoglobus***
Seawater, 2 km distance from active zone	0	0	0	0

Metallic gray slick floating in patches	10	1×10^3	1×10^3	10
Seawater, green discoloration, strong odor of H_2S, 1 km distance from active zone	+	+	+	+
Seawater, green discoloration, strong odor of H_2S, 0.3 km distance from active site	0	0	+	0
Lava rocks with orange-red precipitates from the active crater wall	0	+	+	+

+ quantitatively determined (≥ 1 cell mL^{-1}); 0, not present

Table 2. Hyperthermophilic members of archaeal genera present in samples during 1989 Macdonald Seamount eruption

As we recognized recently, a further type of submarine high temperature environment is provided by active sea mounts. Close to Tahiti, there is a group of active sea mounts which represents hot spot volcanoes situated upon the Pacific tectonic plate. At a depth of 1500 meters, there is the summit crater of Teahicya Seamount which abunds in ferric iron hydroxide minerals and harbours extensive hydrothermal venting systems. In the same area, there is another huge abyssal volcano, Macdonald Seamount (28°58.7' S; 140°15.5'W) the summit of which is situated approximately 40 meters below the sea surface. In 1989, during a French-German expedition, just when we approached the Macdonald area, the seamount began to erupt.

We, therefore, were able to explore for the first time an erupting submarine seamount and its surroundings for the presence of hyperthermophilic archaea. On the first day of eruption (24.01.1989) we took hydrocast samples about 2 to 4 kilometers away from the active site. pH of the sea water was between 8.30 and 8.02 and methane concentration varied between 41.5 and 62.2 nanoliters per liter, which are normal values for surface open sea water. After one quiescent day eruptions began again, and large patches of greyish slick of metallic appearance floated at the surface. Some of this material was sampled. Next day there were further strong eruptions, interrupted by periods of silence. Hydrocast samples were taken from water showing green discoloration at the surface. They had abnormally low pH values of around 6 and extremely high methane concentrations of up to 7800 nanoliters per liter, indicating that they came from the submarine plume of the eruption. On the same day during a more quiet period, sampling within the active crater was attempted by the submersible *Cyana*. Some lava rocks with orange-red hydrothermal precipitates from the crater wall were collected, but sampling ended when the volcano erupted again.

No hyperthermophiles could be enriched from seawater samples taken on the first day at a distance of two kilometers from the active zone. In contrast, samples taken from the floating metallic grey-looking slick, the sea water from the submarine eruption plume, and from rocks from the active crater contained high concentrations of viable hyperthermophiles (Table 2). As expected, none of the hyperthermophiles enriched was able to grow at 60° C or below, and therefore within ambient sea water. This demonstrates the presence of these organisms in active seamounts and their release during submarine volcanic eruptions.

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Biographical Sketch

Karl O. Stetter was born in Munich, Bavaria (Germany) in July 1941. He obtained a PhD in microbiology from the Technical University of Munich in 1973 and an “habilitation” in microbiology and botany from the Ludwig-Maximilians-University of Munich in 1977. He was successively assistant professor and lecturer in this university. In 1980, he became full professor of microbiology and head of the department of microbiology at the University of Regensburg where he is still working now. Since 1989, he is a member (Professor above-scale) of the Faculty of Life Sciences and of the Institute of Geophysics and Planetary Sciences at the University of California at Los Angeles (UCLA). He is also the cofounder of DIVERSA in San Diego, USA (a biodiversity-based biotech-company).

The major research topic of Karl Stetter and his team is the isolation and characterization of extreme heat-loving (hyperthermophilic) microorganisms, which still at present thrive under conditions that may resemble those of the primitive Earth. Their research focuses on growth conditions and biochemical features of novel isolates with cultivation as a fundamental prerequisite. A very recent target of Prof. Stetter is the cultivation and laboratory studies of novel thermophilic protozoa and the exploration of the upper temperature border within the eukaryotes.

His work and research have been awarded on several occasions, notably in 1994 when Prof. Stetter received the Gold Medal Lecture, from the International Institute of Biotechnology, London. He is also the author of about 300 publications.