Hyperthermophiles: optimum for them is extreme for the rest

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Abstract. This paper is a minireview on Hyperthermophiles, which were discovered in 1965 in hot springs in Yellowstone National Park, Wyoming, USA. They are a peculiar subset of thermophilic microorganisms, that live in extremely hot environments, at temperatures of 60°C and above. A remarkable heat-tolerant hyperthermophile is the recently discovered Strain 121 which has been able to double its number during 24 hrs in an autoclave at temperature of 121°C. The protein molecules in the hyperthermophiles exhibit high thermostability, meaning that they can maintain structural stability and function at elevated temperatures. Understanding the Hyperthermophiles could be useful for the industrial bioprocesses development and astrobiology studies. They are important also for understanding the origins of living organisms, for understanding the evolution and adaptation at the molecular level.
Key Words: Hyperthermophiles, Archaea, bacteria, hot environments, resilient microorganisms.

Hyperthermophiles are a peculiar subset of thermophilic microorganisms, polyphyletic, that live in extremely hot environments, at temperatures of 60°C and higher. Generally, their optimal temperature is above 80°C. Most of the hyperthermophiles are classified within Archaea (Arachaebacteria), although some bacteria are able to tolerate temperatures of around 100°C, as well. For this reason, such species/strains of bacteria are included in this group of hyperthermophiles. Several strains of bacteria can live at temperatures higher than 100°C in the sea where water does not boil because of the high pressure at those large depths. Living in high temperatures environments is not all about these microorganisms. They are also able to withstand other environmental extremes such as high levels of acidity (extremely low pH values) or high doses of irradiation.

Hyperthermophiles were discovered in 1965 by the scientist Thomas D. Brock, in hot springs in Yellowstone National Park, Wyoming, USA (cited by Seckbach et al 2013). Since then, more than seventy species/strains have been reported (wikipedia.org). It is difficult to discriminate between a new species and a new strain in the case of microorganisms. The most resilient hyperthermophiles discovered so far live on the superheated surfaces of the wall of deep-sea hydrothermal vents, requiring temperatures of at least 90°C for survival.

A remarkable heat-tolerant hyperthermophile is the recently discovered Strain 121 which has been able to double its number during 24 hrs in an autoclave at temperature of 121°C. In fact, its name stands for the experimental level of the temperature. At present, the record is held by *Methanopyrus kandleri*, its growth temperature being 122°C (wikipedia.org).

Although no hyperthermophiles have yet been found living at temperatures higher than 122°C, their existence is not excluded. Strain 121 survived being heated to 130°C for two hours, but was not able to reproduce until it had been transferred into a fresh growth medium, at a relatively cooler 103°C. However, based of theoretical principles, scientists believe that is less likely that microbes could survive at temperatures higher than 150°C due to the fact that the cohesion of DNA, RNA, and other vital molecules begins to break down at that temperature.

Early research into hyperthermophiles speculated that their genome could be characterized by high guanine-cytosine (GC) content; however, recent studies show that there is no obvious correlation between the GC content of the genome and the optimal environmental growth temperature of the microorganisms (Zheng & Wu 2010).

The protein molecules in the hyperthermophiles exhibit high thermostability, meaning that they can maintain structural stability and function at elevated temperatures. Scientists show that such proteins are homologous to their functional analogues in organisms which thrive at lower temperatures, but have evolved to exhibit optimum functionality at much higher temperatures. Most of the low-temperature homologues of the hyperthermostable proteins would probably be denatured at temperatures above 60°C. Such highly thermostable proteins are often commercially important, as chemical reactions proceed faster at high temperatures (Saiki et al 1988).

Another peculiarity of these microbes is the cell membrane composition. The membrane contains high levels of saturated fatty acids, having the role to retain the membrane shape at elevated temperatures.

There is a mixture of traits in these hyperthermophiles, of which some structural, others functional or interconnected, and acquired, selected and repeatedly tested through the millions of years.

The Most Important Hyperthermophiles. Most of these creatures of the group are Archaea (see a synthetic classification of the microorganisms in Figure 1), but there are also some bacteria, more exactly Gram-negative Bacteria.

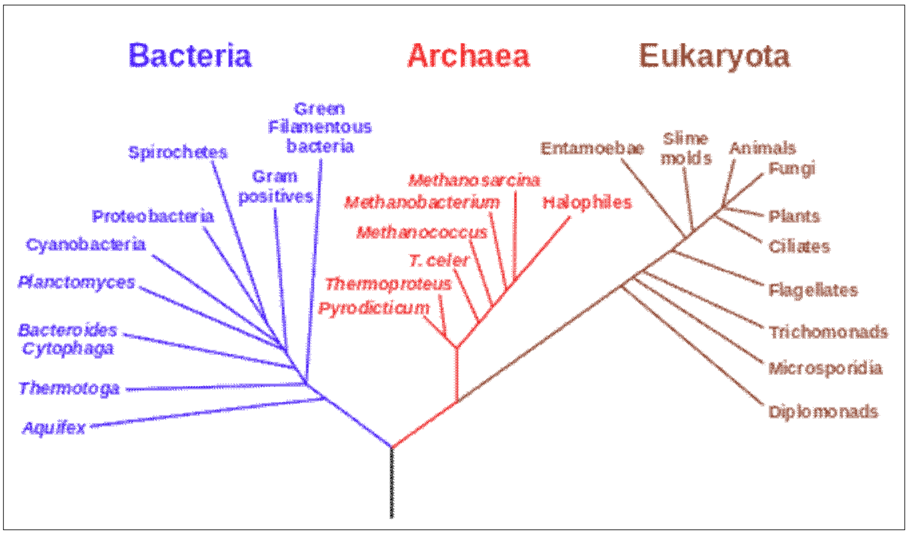

Figure 1. A synthetic classification of the living organisms based on phylogenetic criteria (https://ro.wikipedia.org/wiki/Procariote).

Archaea

Geogemma barossii. Strain 121 is an archaeon living at 121°C in the Pacific Ocean. It is included in *Geogemma barossii*, but in literature it is best known as Strain 121.

Pyrolobus fumarii was first discovered in 1997 in a black smoker hydrothermal vent at the Mid-Atlantic Ridge, setting the upper temperature threshold for known life to exist at 113°C (Blöchl et al 1997).

Pyrococcus furiosus is an archaebacterium which thrives at 100°C. It was first discovered in Italy near a volcanic vent.

Archaeoglobus fulgidus is a sulphur-metabolizing organism. Its metabolic pathway is a sulfur-oxide reduction pathway. Known Archaeoglobales are anaerobic microorganisms, most of which are hyperthermophiles. Most of these Archaeoglobales are found in hydrothermal environments such as hot springs, oil wells, and hydrothermal vents (Lapaglia & Hartzell 1997). *Archaeoglobus* species form iron sulphide which is known to cause corrosion of iron and iron alloys. *A. fulgidus* have cells that are cocci with a glycoprotein envelope and monopolar flagella.

Methanocaldococcus jannaschii is a well known hyperthermophilic methanogenic archaebacterium in the class Methanococci. It was the first archaebacterium to have its complete genome sequenced (Bult et al 1996). The genome sequencing revealed that there are many genes unique to the group Archaea. Many of the synthesis pathways for methanogenic cofactors were worked out biochemically in this archaean microorganism (White 2001), as were several other archaeal-specific metabolic pathways.

Aeropyrum pernix is somewhat different from the microorganisms presented above. It was the first strictly aerobic hyperthermophilic archaebacterium discovered, and was originally isolated from heated oceanic sediments and venting water collected in 1996 from a solfataric vent at Kodakarajima Island in Kyūshū, Japan (Sako et al 1996). Its complete genomic sequence was published in 1999 and its genome size is 1,669 kb, with 2,694 possible genes detected (Kawarabayasi et al 1999). All of the genes in the Krebs cycle were found except for that of oxoglutarate dehydrogenase complex. In its place, the genes coding for the two subunits of 2-oxoacid:ferredoxin oxidoreductase were identified. The cells of *A. pernix* are cocci and have a diameter of approximately 1 µm. The envelope surrounding the cells is wide of about 25 nm. *A. pernix* grows at temperatures between 70 and 100°C (the optimum temperature being between 90 and 95°C), at pH 5-9 (the optimum being the neutral value, pH 7), and at a salinity of 1.8-7% (the optimum salinity being 3.5%). The growth of *A. pernix* is not observed at unsuitable values of 68 or 102°C. Below the value of 1.5% salinity, cells lyse by low osmotic shock. The cells of *A. pernix* are sensitive to the antibiotic chloramphenicol and insensitive to antibiotics such as: ampicillin, vancomycin, and cycloserine. The species grows well on proteinaceous substances, with a doubling time under these conditions of about 200 minutes (Sako et al 1996).

Sulfolobus is one of the genera in the family Sulfolobaceae. It belongs to the Archaebacteria domain. *Sulfolobus* species grow in general in volcanic springs with optimal growth occurring at pH 2-3 and temperatures of 75-80°C. They are both acidophiles and thermophiles in the same time. *Sulfolobus* cells are irregularly shaped and flagellar. Its proteins are of interest for biotechnology and industrial use due to their thermostable nature. The enzymes of *Sulfolobus* maintain their function at high temperature and they are for this reason of great interest and importance for industrial bioprocesses. One application is the creation of artificial derivatives from *S. acidocaldarius* proteins, called affitins. Intracellular proteins are not necessarily stable at low pH however, as *Sulfolobus* species maintain a significant pH gradient across the outer membrane. Microorganisms in the order Sulfolobales are metabolically dependent on sulfur: no matter if heterotrophic or autotrophic, their energy comes from the oxidation of sulfur and/or cellular respiration in which sulfur acts as the final electron acceptor. For example, the species *S. tokodaii* is known to oxidize hydrogen sulfide to sulfate, the bioprocess being located inside the cell. Due to their industrial importance, many species of the genus *Sulfolobus* are sequenced of their genome is planned to be sequenced. The complete genome has been sequenced for *S. acidocaldarius* DSM 639 (having 2,225,959 nucleotides) (Chen et al 2005), *S. solfataricus* P2 (having 2,992,245 nucleotides) (She et al 2001), and *S. tokodaii* strain 7 (having 2,694,756 nucleotides) (Kawarabayasi et al 2001).

Methanopyrus kandleri is a hyperthermophilic, halotolerant, methanogenic archaeon. The most representative, Strain 116 thrives at 80-122°C in a Central Indian Ridge. The species *M. kandleri* seems to have a significant importance for taxonomy and phylogeny. Currently the only species in the genus *Methanopyrus*, *M. kandleri* is thought to be part of a separate lineage from all other known methanogens, possibly a lineage that diverged extremely early on, close to the time that the kingdoms of life were themselves diverging (Kurr et al 1991).

Thermoplasma acidophilum is an archaeon that was originally isolated from a self-heating coal refuse pile, at pH 2 and 59°C. It is situated at the limit between

Hyperthermophiles and Thermophiles. *T. acidophilum* is highly flagellated and grows optimally at 56°C (133°F) and pH 1.8. The size of a cell is about 1 μm. It lacks a cell wall and the cell membrane is exposed directly to exterior. The species shows various cell shapes depending on growth conditions and stages of development. Its genome has been sequenced entirely (Ruepp et al 2000).

Gram-negative Bacteria

Geothermobacterium ferrireducens is a species of bacteria which thrives in 65–100°C in Obsidian Pool, Yellowstone National Park, USA. The species is hyperthermophilic, anaerobic, rod shaped and motile by means of monotrichous flagellation. An interesting feature is its optimal temperature, that is between 85-90ºC, the highest temperature of any bacterial organism (Kashefi et al 2002).

Aquifex aeolicus grows best in water with temperature of 85-95°C, and can be found near underwater volcanoes or hot springs. The species requires oxygen to survive (though it can grow in levels of oxygen of only 7.5 ppm), and its process of respiration produces water as a byproduct. Members of this species tend to form large cell conglomerations, of up to 100 individual cells. This bacterium was discovered around islands north of Sicily (wikipedia.org).

The genome of *A. aeolicus* has been successfully mapped (Deckert et al 1998). This was made easier by the fact that the length of the genome is only about one third of the length of the genome for *Escherichia coli*. Comparison of the *A. aeolicus* genome to other organisms showed that around 16% of its genes originated from the Archaebacteria domain. This species has the smallest genome of any known non-parasite organism. Its total genome is 1.5 Mb long and is estimated to encode about 1512 genes. Additionally, it contains one extrachromosomal element.

This bacterial species is a good candidate for biotechnological use due to its high stability against oxygen and high temperature. This aspect can be useful for the perspective of improving industrial processes (Guiral et al 2012).

Genus Thermotoga is a hyperthermophilic organism that is a member of the order Thermotogales. There are more species of interest for researchers in the genus *Thermotoga*, especially *Thermotoga maritima*. *T. maritima* is a non-sporulating, rod shaped, gram-negative bacterium. As an anaerobic fermentative chemoorganotrophic organism, *T. maritima* catabolizes sugars and polymers and produces carbon dioxide and hydrogen gas as byproducts of fermentation (wikipedia.org). *T. maritima* is also able to metabolize cellulose as well as xylan, yielding H_2 that could potentially be used as an alternative energy source to fossil fuels (Nelson et al 1999). Additionally, this microbial species is able to reduce iron [Fe(III)] to produce energy by anaerobic respiration. Several flavoproteins and iron-sulphur proteins have been detected as possible electron carriers for use during the cell respiration (Nelson et al 1999). However, when growing with sulfur as the final electron acceptor, no ATP is produced. On the other side, this process eliminates inhibitory H_2 produced from fermentative growth (Nelson et al 1999). All together, these characteristics and abilities indicate that *T. maritima* has become resourceful and capable of metabolizing a lot of substances in order to carry out its life processes.

Conclusions. Hyperthermophiles are a peculiar subset of thermophilic microorganisms, polyphyletic, that live in extremely hot environments, at temperatures of 60°C and higher. Knowledge on Hyperthermophiles is highly useful for the development of industrial bioprocesses and astrobiology. They are important also for understanding the origins of living organisms, for understanding the evolution and adaptation at the molecular level.

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