THERMOPHILY

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Contents

Summary

Thermophilic microorganisms have long been a source of fascination for scientists, since they can grow in high temperatures, currently up to 113 °C, which were unthinkable only short time ago. In this article we describe the main natural extreme environments characterized by high temperature, and colonized by microorganisms. This covers environments such as freshwater alkaline hot springs, acidic solfatara fields, and anaerobic geothermal mud and soils. The community structure, in terms of available energy sources and representative autotrophic and heterotrophic microorganisms, is discussed for each type of habitat. The diversity and distribution of major bacterial lineages, such as *Thermus* and *Aquificales*, as well as key archaeal species, are discussed in the context of evolution and the mechanisms for generation of new species

in extreme habitats. All accepted species of thermophilic bacteria with maximum growth temperatures above 65 °C are listed according to their phyla, with information on the main physiological parameters that are characteristic for the species.

1. Introduction

The phenomenon of thermophily, as we know it today, was established by Thomas Brock through his extensive and pioneering studies in Yellowstone National Park (Wyoming, USA) from 1968 to 1978. It is now more than 30 years since he discovered *Thermus aquaticus*, the first isolated organism shown to grow above 70 °C. Ever since, thermophiles and extremophiles, in general, have attracted a constantly increasing level of interest. Before 1970, several thermophiles had been isolated that could grow up to 70 °C, and much research had been done on strains of *Bacillus stearothermophilus*, including the isolation and study of thermostable enzymes.

Brock also isolated *Sulfolobus acidocaldarius* and *Thermoplasma acidophilum*, the first representatives of the thermoacidophilic archaea. The discovery of archaea as a third domain of life, in addition to eukarya and bacteria, was a major scientific event. It came at a time when microbiology was going through a slowly evolving period, and the general feeling was that all the major scientific discoveries had already been made. The next big wave in thermophile research started in 1981 when Karl Stetter and Wolfram Zillig isolated *Thermoproteus*, the first anaerobic, extreme thermoacidophile, from Icelandic hot springs. Since then, several new species and genera have been isolated, including the landmark discoveries by Karl Stetter and his colleagues of *Pyrodictium*, the first organism to grow optimally at 105 °C, and *Pyrolobus*, which has a maximum growth temperature of 113 °C—the highest growth temperature yet recorded for any living organism.

The research topics of thermophily are still expanding into evermore diverse fields, but it is molecular-biology methods, including whole genome sequencing and bioinformatics, that have had the most profound impact on our current understanding of thermophily.

1.1. Thermophily and Geochemical History

Life has existed on earth for more than 3.7 billion years, and it may already have been present as early as 4 billion years ago. Evolution has since led to extensive diversification in microorganisms. The present-day microbial diversity is so great that it is now generally accepted that we have yet only explored a small fraction of it, and that much discovery work still remains ahead.

Microorganisms have certainly played a major role in the evolution of the biosphere and the creation of the earth’s crust as we know it today. It is thought that the earth originated from a collision of meteorites or by a collection of space dust 4.6 billion years ago. This matter became very hot and melted, resulting in the release of gases and water, which condensed on the surface. The primitive earth’s atmosphere probably contained reduced chemicals, such as methane and ammonia. It also contained CO_2, but no oxygen. The surface of the earth must have been very hot in the beginning and then slowly cooled. The length of the cooling period is unknown, but it has generally been assumed that the temperature was close to what it is today when life started on the planet. The oldest rocks known to contain structures that can be interpreted as microfossils are about 3.4–3.5 billion years old. Stromatolite structures found in 2.8 billion-year-old limestone are taken as evidence for the existence of cyanobacteria at that time.

Nowadays we can put together a set of strong arguments for the hypothesis that the first living organisms on earth were thermophiles. There is no geological evidence that argues against this. In fact, some geologists now feel that even 3 billion years ago it was much hotter than it is now. The average temperature fluctuation from one place to another on the present-day earth is at least 80 °C, therefore, we can just as well propose that 3 billion years ago the earth had thermophilic temperatures of around 80 °C, instead of more mesophilic temperatures.

Furthermore, we can state that hot springs have always existed on earth, and they may have been much more widespread in the past than they are now. It is, therefore, also conceivable that life originated in a hot spring. The reduced chemical conditions in the hot soils of present solfatara fields probably correspond well with those of the early earth.

Another strong argument in favor of this hypothesis is the fact that all of the deepest and most slowly evolving branches on the tree of life are represented primarily by extreme thermophiles. The prevalent sulfur-based metabolism of the most thermophilic, fermentative and sulfur-utilizing, anaerobic archaea corresponds very well with the expected environment of the early earth. All this seems to support the hypothesis of thermophilic ancestry for both archaea and bacteria, and perhaps for eukaryotes as well.

In the context of this discussion it is interesting to note that concurrent with increasing biological complexity there is a stepwise lowering of the maximum growth temperature for the organisms (see Table 1). The most thermophilic archaea are fermentative, sulfur-respiring, or microaerophilic, tolerating only extremely low oxygen tension; the most thermophilic bacteria have similar metabolisms. We first find fully aerobic metabolism among the extremely acidophilic archaea and among the more common bacterial thermophiles, such as *Thermus*. Anoxygenic and oxygenic photosynthesis first occurs in bacteria at 70–73 °C. The first heterotrophic eukaryotes are at 60–62 °C, but the highest temperature for photosynthetic eukaryotes is at 55–58 °C, and, finally, for more complex eukaryotes, at 45–50 °C.

Organism group	**Maximum growth temperature °C**
Animals	
Fish	38
Insects	45–50
Crustacea	48–50
Plants	
Vascular plants	45

Bryophytes	50
Eukaryotic microorganisms	
Protozoa	56
Algae	55–60
Fungi	60–62
Prokaryotes	
Cynaobacteria	70–72
Green bacteria	70–72
Bacteria	95
Archaea	113

Table 1. Maximum growth temperature for main groups of organisms

The current model assumes that life was first created at relatively low temperatures, and evolved simultaneously into more extreme environments on the one hand, and to form more complex structures on the other. Perhaps we can make a different model, assuming that life first arose at thermophilic temperatures, low enough to allow the minimum components necessary for a living cell to remain stable. Then as soon as the favorable low temperatures had been reached, more and more complex structures quickly evolved, allowing new metabolic functions to be established. The temperature versus complexity order discussed above can be interpreted in support of the latter model.

1.2. Definitions and Terminology

Temperature is certainly the most important variable in the environment. The classification of living organisms based on their relation to temperature has, therefore, always been considered as one of the most basic elements of biological classification and systematics. Organisms that grow optimally at 45–55 °C are ubiquitous, but those with an optimum temperature above 60 °C are, in general, associated with permanently hot places, such as areas of geothermal activity. Many thermophiles, in particular spore-forming organisms, can however be isolated from many nongeothermal areas, including solar-heated soils and manmade environments, such as composts and domestic or industrial heating systems.

Microorganisms have traditionally been divided into three main groups in this respect: psychrophiles, mesophiles, and thermophiles. The temperature range of every organism over which it can grow and reproduce has been defined in terms of three temperature points—often called cardinal temperatures—that are characteristic for each microbial species or strain. These are: T_{min}, the lowest measurable growth temperature; after which the growth rate will first increase almost linearly for about 15–25 °C, up to the optimum growth temperature (T_{opt}); after which it usually falls abruptly for the next 5–10 °C, ending at T_{max}, above which no growth occurs. The so-called optimal temperature may not really be the optimal temperature for the microorganisms because it results from kinetic effects (Arrhenius's law) which do not have to do with the optimum physiological state of the organism. Attempts have been made to define different groups according to cardinal temperatures, but owing to overlaps and exceptions this has been difficult.

Brock suggested a definition of a thermophile boundary at 55–60 °C, based on two main arguments. First, temperatures below this boundary are common in nature, but higher temperatures are mainly associated with geothermal activity or some very special situations. Second, no eukaryotes are known to grow above this boundary; therefore, it would be an exclusively prokaryotic world. The thermophilic range above the boundary will then be exactly half the currently known temperature span of life (–10 °C to 113 °C).

The thermophilic range still needs to be divided further, and Stetter has used the terms "extreme thermophile" for organisms growing up to 80 °C and "hyperthermophile" for those that can grow optimally above 80 °C. These terms are now frequently used and seem generally to be accepted. For the present discussion we treat thermophilic bacteria as all those that can grow at temperatures above 65 °C (see Table 6).

2. Habitats and Ecology

2.1. Diversity of Thermal Environments

Geothermal activity on earth is mainly connected with tectonic activity where plates are moving, either drifting apart or colliding against each other. Such areas are characterized by volcanic activity and in many places geothermal areas on the surface. Geothermal areas are extremely varied in terms of geology and chemistry, but belong mainly to two categories: first, the solfatara type, characterized by fields with much sulfur, acidic soils, acidic hot springs, and boiling mud pots; second, the neutral-alkaline type, characterized by freshwater hot springs and geysers, which are neutral to a1kaline in pH.

Natural geothermal areas are found in all parts of the globe associated with tectonic activity, but are usually concentrated in small places. The best known and biologically most studied geothermal areas are in Iceland, North America (Yellowstone National Park), New Zealand, Japan, Italy, and the former Soviet Union. If we take Iceland as an example, it is located on the Mid-Atlantic Ridge where the American and the European plates are moving away from each other; and it is considered as one of the earth's hot spots because it sits on a mantle plume, where huge amounts of lava and geothermal energy come to the surface, resulting in frequent volcanic eruptions. Geothermal areas in Iceland cover in total more than 500 km^2, which is over 0.5% of the total surface of the country.

The two different classes of geothermal area are the result of geological differences in the heat source. One type is called high temperature fields, primarily located within active volcanic zones and having a magma chamber at a depth of 2–5 km as a heat source. It is usually characterized by water temperatures of 150–350 °C at a depth of 500–3000 m and by emissions of steam and volcanic gases on the surface. The gas is composed primarily of N_2 and CO_2, but H_2S and H_2 can be up to 10% each of the total gas fraction. Also, traces of methane, ammonia, and carbon monoxide are often found. Because of the weak acids CO_2 (pK = 6.3) and H_2S (pK = 7.2), the pH of the subsurface steam is near neutrality. On the surface the H_2S is oxidized chemically and biologically, first to sulfur and then to sulfuric acid. This lowers the pH, causing corrosion of the surrounding rocks and formation of the typical acidic mud of solfatara fields. The pH often stabilizes around 2–2.5 where sulfuric acid ($pK_2 = 1.92$) is the effective buffering agent. Because of the high temperature, there is little water that comes to the surface and the hot springs are mostly steam holes or fumaroles. These areas are usually unstable, and the individual openings often disappear or move to another location within the geothermal field. The top few centimeters of soils in such solfatara fields are also very acidic, but the subsurface soils are less acidic than the hot springs themselves. The soils are usually very dense and moist, and are reduced by high sulfide. Only the top 1–2 cm are oxidized and acidic, but below this, the pH may be 5–6.

The other types of geothermal areas are called low temperature fields and are located outside the volcanically active zones. They are heated by deep lava flows or by dead magma chambers. The water temperature at a depth of 500–3000 m is usually below 150 °C. Groundwater percolates into these hot areas, warms up, and returns to the surface containing dissolved minerals, such as silica, and some dissolved gases, mainly CO_2. Usually, there is little H_2S in such fluids. Also, the subsurface pH here is near neutrality, but there is usually much more water and little sulfide so its surface oxidation has no effect on the pH. On the surface, however, the CO_2 escapes and the silica precipitates, both resulting in increased pH. The pH is often stabilized at 9–10 where silicate ($pK_n = 9.7$) and carbonate ($pK_2 = 10.25$) start to act as effective buffering agents. Since these areas are located outside the volcanically active zones, they are geologically rather stable, and the individual hot springs are very constant in both temperature and water flow. However, they are often disrupted, or new ones created, during periods of increased earthquake activity. Both types of geothermal field are also found on the seafloor, adding salinity and even sharper temperature gradients to the other factors. Also, the sulfide gets oxidized here to sulfur and sulfuric acid, but owing to the huge water mass around, it cannot affect the pH to the same extent as in the terrestrial fields.

Stable hot natural habitats other than geothermal are very few, burning coals perhaps the only one. Solar-heated ponds and soils are, of course, common; and biological self-heating in composts, hay, litter, or manure may cause quite high temperatures and even spontaneously ignition. These are, however, transient ecosystems, and there are mainly rapidly growing spore formers that can take advantage of such biotopes. Several manmade constant hot environments have been created. These include hot water pipelines and heat exchangers in homes and factories, burning coal refuse piles, and other types of mining heap, as well as thermophilic waste treatment plants. Numerous processes in the food and chemical industries use aqueous evaporation or extraction, which is run at high temperatures and often creates ideal conditions for the growth of thermophilic microorganisms. Several well-known thermophiles have been isolated from such manmade systems, and some have not even been found elsewhere.

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Biographical Sketches

Gudmundur O. Hreggvidsson is an Assistant Professor at the University of Iceland (from 1999) where he lectures in microbial biotechnology and microbial ecology. He is also the Vice President of Research and Bioinformatics at Prokaria Ltd. He was a research scientist working on thermophilic bacteria at the Technological Institute of Iceland from 1990 to 1998. He has a B.Sc. in Biology from the University of Iceland, 1983, and a Ph.D. in Molecular Biology from the University of Edinburgh, UK, 1993. He is the coauthor of about 25 papers in refereed international journals, and has five international patents applications in various fields of thermophilic microbiology and biotechnology.

Jakob K. Kristjansson is Research Professor in Biotechnology at the University of Iceland and President and founder of Prokaria Ltd., a company currently with 40 employees and focusing on GENEMINING™ for new enzymes from natural microbes. He was the Head of the Biotechnology Department, Technological Institute of Iceland (1985–1998), Associate Professor of Microbiology (1986–1998), and Research Professor in Biotechnology (from February 1998) at the University of Iceland. He has a B.Sc. in Biology (University of Iceland, 1976), and a Ph.D. in Biochemistry (Brandeis University, USA, 1980).

He was the Alexander von Humboldt Fellow at Philipps-Universität, Marburg, West Germany in 1981/1982. He has published over 60 papers and book chapters, and edited a book on thermophilic bacteria. The main activity of his research team has been on the biotechnology of thermophiles isolated from the numerous hot springs in the geothermal fields in Iceland. A thermophilic bacterium, *Caldicellulosiruptor kristjanssonii*, was named in his honor in 1999.