THERMOPHILIC BACTERIA

Jakob K. Kristjansson

CRC PRESS

Thermophilic Bacteria

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CRC Press, Inc.
Boca Raton Ann Arbor London

Library of Congress Cataloging-in-Publication Data

Thermophilic bacteria / editor, Jakob K. Kristjansson.
p. cm.
Includes bibliographical references and index.
ISBN 0-8493-5239-8
1. Thermophilic bacteria. I. Kristjansson, Jakob K.
QR84.8.T44 1991
589.9'5—dc20 91-19399
CIP

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Direct all inquiries to CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, Florida 33431.

International Standard Book Number 0-8493-5239-8

Library of Congress Card Number 91-19399

Printed in the United States of America 1 2 3 4 5 6 7 8 9 0

PREFACE

Thermophiles have been of interest for a long time both to scientists and to the general public. This is understandable since these organisms can live and thrive under extreme conditions which most living organisms cannot tolerate.

The present wave of interest in thermophiles goes back to the pioneering work of Thomas Brock in the 1970s. It has been further strengthened by the discovery of the Archaebacteria and especially by the work of Stetter and Zillig and colleagues on the hyperthermophilic anaerobic archaebacteria, some of which can grow in boiling water and even up to 110°C.

Because none of the thermophiles belonging to the usual bacteria "the eubacteria" can grow above 90°C, it is understandable that the former, most fascinating microorganisms, have received much of the attention in recent years.

A great deal of new information on the "eubacterial" thermophiles has been generated in a large number of laboratories throughout the world. This research has focused on both basic and applied aspects of thermophily. Thermophiles are believed to have a great potential in biotechnology and some products are already on the market. Thermostable DNA polymerases, for example, are essential for the well-known PCR technology.

The purpose of this book is to collect all the new information on the thermophilic (eu)bacteria and to present the current status of research on those microorganisms which seem to dominate the thermophilic world below 90°C. The lower temperature limit for this book was defined as organisms that could grow at 60 to 65°C, although there are some exceptions to this.

Since the discovery of the archaebacteria there have been intense discussions on the taxonomy of the procaryotes. Many nomenclatural changes have been made and new names and classes proposed. Recently, Woese, Kandler, and Wheelis (*Proc. Natl. Acad. Sci. U.S.A.*, 87, 4576, 1990) have recommended that the terms "Archaebacteria" and "Eubacteria" should no longer be used, but instead "Archaea" and "Bacteria". This is based on the arguments that "Archaea" have so little in common with "Bacteria", except both being procaryotes, that they should not be called archae-bacteria. By the same token, there is no need to define the "Bacteria" as the "true" (eu-) bacteria since they would then be the "only" bacteria, as it was in the past.

Many eminent microbiologists have already chosen to follow this new convention and use "Archaea" and "Bacteria". As the editor of this book I decided to do the same and, therefore, the title of the book is *Thermophilic Bacteria,* instead of *Thermophilic Eubacteria.* I realize that many may disagree with this, but a choice had to be made and for the sake of uniformity all the contributors agreed to use the new convention for this book.

My work as editor was in part supported by the "Promotion Award" from the Icelandic National Research Council. I thank all of the authors for their extensive contributions and I am confident that *Thermophilic Bacteria* will be a useful book for all those with an interest in basic and applied aspects of thermophilic microbiology.

Jakob K. Kristjansson
Reykjavik, July 1991

THE EDITOR

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Dr. Kristjansson is a member of the American Society for Microbiology, the German Society for Microbiology, the Icelandic Academy of Sciences, the Icelandic Society for Microbiology and numerous other Icelandic societies. He has served on several committees for the Icelandic National Research Council, the Nordic Council of Ministers and the Nordic Industrial Fund. He is now a member of the board of the Icelandic National Research Council.

Dr. Kristjansson has received grants from the Fulbright Foundation, the American Scandinavian Foundation, the Jessie Smith Noyes Foundation and the Alexander von Humboldt Foundation. He received "the Promotion Award" of the Icelandic National Research Council when awarded for the first time in 1987. He has held numerous research grants from the Icelandic Science Foundation, the Icelandic Research Council, the Research Fund of the University of Iceland, the Nordic Fund for Technology and Industrial Development and private industry. He has published more than 40 papers. His current research interests include the microbiology and biochemistry of thermophiles and thermostable enzymes.

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Chapter 1

THERMOPHILIC BACTERIA

Jakob K. Kristjansson and Karl O. Stetter

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I. INTRODUCTION

Thermophilic microorganisms have been known for a long time but usually considered as some sort of biological eccentrics. Thermostable microbes and spores have previously been looked at as pests in the food processing and canning industry. In the past we generally learned that living organisms and also their individual components such as proteins are killed or denatured by heating. It is, therefore, very difficult to comprehend that some organisms do not only survive at high temperatures, but actually thrive in boiling water.[1,2]

The possible exploitation of the thermostable properties of thermophiles has been recognized for some time but has only recently really started to be realized in industry.[3-5]

Many enzymatic or microbiological industrial processes are already run at a high temperature and those that are not could, in many cases, benefit from increased heating if not prevented by some unstable components in the process. The main advantages of increased temperature are generally higher reaction rates, higher solubility of most chemicals, and increased fluidity and diffusion rates. The early notion that high temperature prevents microbial contamination is only partly correct. It does, in fact, prevent growth of most environmental mesophilic microorganisms and pathogens but thermophilic microbes, especially spore formers, are surprisingly common contaminants in many processes which are run at an elevated temperature.[4,6]

The main interest in thermophiles in recent years has been on two fronts. On the scientific front has been the discovery of many new extremely thermophilic archaea, some of which can grow at 100°C and even above.[2] On the biotechnological front has been the realization that thermophilic microorganisms can serve as excellent sources for more thermostable biocatalysts than are presently available.[4,7] More stable biocatalysts can open up many new possibilities that were not known before, for example, in bioorganic synthesis and in biosensors.

The discovery of archaea as a *third domain of life* in addition to eucarya and bacteria (previously named archaebacteria, eubacteria, and eucaryotes) was a major scientific happening.[8,8a] It came at a time when scientific microbiology was going through a slowly evolving period. The scientific community had the general feeling that all major scientific discoveries had already been made and that the time of great exploring missions was really over. This discovery, therefore, created a sensation in biological science, especially among microbiologists.

The discovery of several new extremely thermophilic and hyperthermophilic bacteria during the last few years, and the fact that almost all of them belonged to the archaea made the area even more exciting.[8a] In view of this, it is not surprising that thermophilic archaea have received much attention in the last few years and that thermophilic bacteria have been overshadowed.

Considerable research work on thermophilic bacteria is still going on, and several new groups have also been discovered among them as well (see Table 4). Fields such as enzymology and molecular biology of thermophilic bacteria are, in fact, much more developed than with archaea, although it is increasing rapidly too.

It was therefore felt that it was time to regroup the thermophilic bacteria and to collect new and relevent information in one book. Several books and reviews with different emphases have been written in the last few years on thermophiles, but a collection on thermophilic bacteria such as this has never been published before.

Bacteria have traditionally been classified according to characters such as morphology and physiology, but it has not been possible to determine the evolutionary relationships between the different bacterial groups. With the entry of natural phylogeny into microbial systematics based on nucleic acid sequence data, it is now possible to classify microorganisms on evolutionary terms as well.[9] Based on this we now look at the procaryotes as two completely unrelated classes, archaea and bacteria.[8a] It has also been possible to identify the main phylogenetic groups within each class.[9,10]

In this book, however, the thermophilic bacteria are classified in a traditional way according to physiology, metabolism, and morphology. The recent recommendation by Woese, Kandler, and Wheelis that living organisms should be organized into three natural domains: the archaea, the bacteria (both belonging to the procaryotes), and the eucarya, is adopted here.[8a] In this chapter the main properties and differences of bacteria and archaea are discussed. The diversity, ecology, and evolution of thermophiles are also briefly reviewed.

II. DEFINITIONS AND TERMINOLOGY

Temperature is one of the most important variables in our 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 systematics. Microorganisms have traditionally been divided into three main groups in this respect, e.g., psychrophiles, mesophiles and thermophiles.

There is a certain useful temperature range for every organism over which it can grow and reproduce; at T_{min}, growth can start and then the growth rate will first increase almost linearly for about 15 to 25°C, up to the optimum growth temperature (T_{opt}), after which it usually falls abruptly for the next 5 to 10°C to T_{max}, above which no growth occurs. These three temperature points are called cardinal temperatures and are characteristic for each microbial species or strain. Many attempts have been made to define different groups according to cardinal temperatures; however, since there is always overlapping and exceptions, this has been very difficult and today there is still no general consensus on these definitions. For the last few years the thermophilic field has been expanding to higher temperatures and many new terms have been suggested.[1,3,11]

It can be said that, traditionally, organisms with T_{max} >50°C have been called thermophilic.[3,11] Brock[12] has suggested a definition of a *thermophile boundary* at 55 to 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 eucaryotes are known to grow above this boundary; therefore, it would be an exclusively procaryotic world. The thermophilic range above the boundary will then be exactly half of the currently known temperature span of life (−10 to 110°C).

The thermophilic range still needs to be divided further, and a relatively simple division might be to define thermophilic and extremely thermophilic procaryotes as those which can grow from 55—60°C to 80—85°C and hyperthermophiles as those which can grow optimally above 80 to 85°C.[4] This division will put all known thermophilic bacteria (except the Thermotogales) in the thermophilic range and the majority of the thermophilic archaea in the hyperthermophilic range (see Tables 3 and 4). All organisms discussed in this book can grow at least at 55°C and are therefore above this boundary; in fact, all except some of the phototrophs can grow up to 65°C (see Table 3 and 4).

The main metabolic groups among thermophilic procaryotes are listed in Table 1 with the maximum growth temperature known for any member within each group. In most cases there is only one species or strain which can grow at that temperature and often there is only one thermophilic member in the group. The T_{max} values in Table 1 are all determined experimentally with pure cultures.

III. CHARACTERISTICS OF THERMOPHILES

A. ARCHAEA

Archaea are arranged differently in Table 1 than bacteria because they are very unique

TABLE 1
The Maximum Temperature for Growth of Main Metabolic Groups of Procaryotes

Organisms	T_{max} (°C)	Ref.
Bacteria		
Actinomycetes	62	13
Photosynthetic		
Oxygenic cyanobacteria	73	14, 33
Anoxygenic green bacteria	70	1, 55
Aerobic		
Chemolithoautotrophic		
Hydrogen oxidizing	80	15
Sulfur oxidizing	80	16
Nitrogen oxidizing	<60	17
Iron oxidizing	55	18
Heterotrophic	85	19
Anaerobic		
Nitrate reducing	80	20
Denitrifying	80	16
Sulfate reducing	85	3, 21
Fermenting		
Clostridia	80	3, 84
Thermotoga	90	22
Archaea		
Halophilic	55	23
Methanogenic	97	24
Sulfur-metabolizing		
Aerobic		
Chemolithoautotrophic	96	25
Heterotrophic	90	26
Anaerobic		
Chemolithoautotrophic	110	27
Heterotrophic	105	28

in terms of ecology and physiology. Halophiles live in a unique habitat of saturated salt solutions where no bacteria are as well adapted to grow. The extreme halophiles are really all mesophilic with only one species that can grow up to 55°C. As a group, however, they are metabolically quite diverse. Some of them can utilize light, via bacteriorhodopsin-driven proton pump, to make ATP. Some are facultative fermentative anaerobes, and one species is a denitrifier.[29]

Methanogenic bacteria are all strict anaerobes utilizing a metabolic pathway, methanogenesis, which is unique and which no eubacterium has acquired.[30] In sulfate-rich anaerobic ecosystems such as marine sediments, the methanogenic bacteria must compete with sulfate-reducing bacteria for substrates, which are mainly hydrogen and acetate.[31,32] In low sulfate anaerobic ecosystems such as fresh-water sediments, on the other hand, they seem to have very little competition for those same substrates and are therefore in a unique position.

Sulfur-metabolizing archaea are metabolically the most diverse group among archaea.[2] Some of them, in fact, do not utilize sulfur compounds as an energy source, but belong to this group phylogenetically based on rRNA sequence data.[9] The *Thermoplasma* have a very special place among archaea.[9] They are wall-less heterotrophs, moderately thermophilic, facultatively anaerobic, and extremely acidophilic.[34] Aerobically they do not use any sulfur compounds for growth but gain energy by reducing sulfur anaerobically.[34] *Thermoplasma*,

TABLE 2
Comparison of Bacteria and Archaea

Character	Bacteria	Archaea
Cell wall components	Murein	Pseudomurein proteins, polysaccharides
Membrane lipids	Glycerol fatty acid esters	Glycerol isopranyl ethers
Square and flat structures	−	+
Endospores	+	−
tRNA "common arm" contains	Ribothymidine	Pseudouridine or 1-methylpseudouridine
Methyonyl initiator tRNA formylated	+	−
Introns in genes	−	+
Eucaryotic RNA polymerase	−	+
Special coenzymes	−	+
Max growth temperature	90°C	110°C
Complete photosynthesis	+	−
Methanogenesis	−	+
Calvin cycle used in CO_2 fixation	+	−

therefore, have a way of life similar to *Acidianus*.[25] Phylogenetically they are far from the other sulfur-metabolizing extreme thermophiles and are closer to methanogens.[9]

The sulfur-metabolizing group of extreme thermophiles can utilize seven different modes of life: aerobic heterotrophy, aerobic sulfur-oxidizing autotrophy, anaerobic heterotrophic sulfur respiration (with formation of H_2S), anaerobic autotrophic sulfur respiration, and anaerobic fermentation of organic compounds. The last group can sometimes use sulfur as an electron sink without gaining energy from it.[35] Some of the sulfur-oxidizing autotrophs can also grow by oxidizing metal ions such as metal sulfides,[36,37] and the recently discovered *Archaeoglobus* is a sulfate-reducing heterotroph.[38,39]

Many methanogens are able to reduce sulfur and produce H_2S. Thus, it seems that metabolism of sulfur compounds in one form or another is very common among archaea.[40]

Long before the recognition of archaea as a phylogenetically coherent group, it had been noted that they had many unique features, such as special lipids and cell walls. This was explained as a special adaptation to the unusual extreme environments in which they were found.

It is now clear that many of these features are common among all archaea; others are only found in one of the three major groups. The main characteristics differentiating them from bacteria are listed in Table 2. Most of these properties seem to be there for evolutionary reasons and cannot be convincingly explained as merely adaptations to special environments. An attempt is made in Table 3 to list all formally published species of thermophilic archaea with T_{max} >65°C as of September 1990. Also listed are T_{opt}, pH_{opt}, and year of isolation (publication). A corresponding summary is made for thermophilic bacteria in Table 4.

B. BACTERIA

It is quite clear that both archaea and bacteria are very diverse in all respects. Thermophilic bacteria can be found in most bacterial metabolic groups although some have only one known thermophilic representative. It is quite clear from Table 1 that many of the bacteria are not adapted to grow at very high temperatures. Most of the thermophilic representatives among the bacteria have an optimum temperature below 75°C, with *Thermotoga* the only one with a temperature optimum at 80°C. Some groups such as iron- and ammonia-oxidizers have no representatives which can grow above 60°C.

TABLE 3
List of Thermophilic Archaea with T_{max} >65°C

Organisms	T_{max}	T_{opt}	pH_{opt}	Year	Ref.
Methanogens — Obligate Anaerobes					
Methanobacterium thermoautotrophicum	75	65	7.4	1972	41
M. thermalcaliphilum		60	7.7	1985	41
Methanothermus fervidus	97	83	6.5	1981	24
M. sociabilis	97	88	6.5	1986	42
Methanococcus jannaschii	86	85	7.0	1983	43
M. thermolithotrophicus	70	65	7.0	1982	43
M. igneus	91	88	5.7	1990	118
Methanogenium thermophilicum	65	55	7.0	1982	44
Methanothrix thermoacetophila		65	7.4	1984	45
Methanopyrus kandleri	110	98	6.5	1991	64
Thermoacidophiles — Obligate and Facultative Aerobes					
Thermoplasma acidophilum	65	60	1.5	1970	46
T. volcanium	67	60	2.0	1988	34
Sulfolobus acidocaldarius	90	75	2.5	1972	26
S. solfataricus	90	87	3.0	1980	47
Acidianus infernus	96	90	2.0	1986	25
A. brierleyi	75	70	2.0	1973	48
Metallosphaera sedula	80	75	3.0	1989	37
Sulfurococcus mirabilis	85	73	2.3	1987	59
Desulfurolobus ambivalens	87	80	2.5	1986	62
Thermoneutrophiles — Obligate Anaerobes					
Thermococcus celer	97	88	5.8	1983	49
T. stetteri	98	88	6.5	1989	117
T. litoralis	96	85	6.0	1990	52
Pyrococcus furiosus	103	100	7.0	1986	50
P. woesei	105	102	6.2	1988	28
Thermoproteus tenax	96	88	5.5	1981	51
T. neutrophilus	97	85	6.8	1986	2, 58
Thermofilum pendens	100	88	5.0	1983	53
T. librum	95	80	6.0	1986	2
Desulfurococcus mobilis	90	85	6.0	1982	54
D. mucosus	97	85	6.0	1982	54
D. amylolyticus	97	91	6.4	1988	63
D. saccharovorans	90	83	6.5	1986	2
Staphylothermus marinus	98	92	6.5	1986	56
Pyrodictium occultum	110	105	6.5	1982	27
P. brockii	110	105	5.5	1983	57
P. abyssi	110	97	5.5	1991	61
Thermodiscus maritimus	98	90	5.5	1983	58
Pyrobaculum islandicum	102	100	6.0	1987	65
P. organotrophum	102	100	6.0	1987	65
Archaeoglobus fulgidus	92	83	7.0	1987	38, 39
A. profundus	90	82	6.0	1990	116

Note: This list was created from available sources as of October 1990.

TABLE 4
List of Thermophilic Bacteria with T_{max} >65°C

Organisms	T_{max}	T_{opt}	pH_{opt}	Year	Ref.
Aerobes — Obligate and Facultative					
Bacillus acidocaldarius	65	58	2.0	1971	68
B. stearothermophilus	75	60	7.0	1920	68
B. caldolyticus	82	72	7.0	1972	68
B. caldotenax	85	80	8.0	1972	68
B. caldovelox	76	65	8.0	1972	68
B. thermoglucosidasius	69	62	7.0	1983	68
B. thermocatenulatus	78	68	?	1975	68
B. schlegelii	80	70	6.5	1979	68
B. flavothermus	72	60	7.5	1982	68
B. tusciae	65	55	4.5	1984	92
Chloroflexus auranticus	70	56	8.0	1974	55
Synechococcus lividus	73	65	8.0	1971	33
Thermothrix thiopara	80	72	6.8	1976	16
Thermus aquaticus	80	70	7.7	1969	69
T. thermophilus	85	72	7.5	1974	19
T. ruber	70	60	7.2	1975	76
T. filiformis	80	73	7.3	1987	77
Thermomicrobium roseum	80	73	8.3	1973	70
Hydrogenobacter thermophilus	77	72	6.8	1980	71
Calderobacterium hydrogenophilum	82	75	6.5	1983	94
Saccharococcus thermophilus	78	70	6.5	1984	79
Rhodothermus marinus	72	65	7.0	1988	80
Thermoleophilum album	70	60	7.3	1976	60, 67
T. minutum	70	60	6.8	1986	82
Acidothermus cellulolyticus	65	55	5.0	1986	93
Anaerobes — Obligate					
Clostridium thermocellum	68	60	5.7	1926	72
C. thermosulfurogenes	75	60	6.0	1983	72
C. stercorarium	70	65	7.3	1983	72
C. thermohydrosulfuricum	78	69	7.2	1965	72
C. thermoautotrophicum	68	58	5.7	1982	72
C. fervidus	80	68	7.3	1987	84
C. thermosaccharolyticum	65	60	6.7	?	96
Desulfotomaculum nigrificans	65	57	7.0	1927	73
Desulfovibrio thermophilus	85	65	7.5	1974	21
Thermoanaerobium brockii	76	67	7.5	1979	74
Thermoanaerobacter ethanolicus	78	69	8.0	1981	75
Thermodesulfobacterium commune	85	70	7.0	1983	81
Acetogenium kuvui	72	66	6.4	1981	78
Thermobacteroides acetoethylicus	75	65	7.2	1981	83
T. leptospartum	71	60	7.5	1988	95
Dyctioglomus thermophilum	80	78	7.0	1985	85
Caldocellum saccharolyticum		68	7.0	1986	86
Thermoanaerobacterium lactoethylicum	75	65	7.0	1989	87
Fervidobacterium nodosum	80	67	7.3	1985	88
F. islandicum	80	65	7.0	1990	97
Thermotoga maritima	90	80	6.5	1986	22
T. neapolitana	90	80	7.0	1988	89
T. thermarum	84	70	7.0	1989	90
Thermosipho africanus	77	75	7.2	1989	91
Acetomicrobium faecalis	77	72	6.7	1987	98

Note: Different definitions or considerations may be used in other chapters of this book. Therefore, some organisms discussed by other authors in this book are not listed in this table.

Since there is so much new data concerning archaea, it is not customary to look at the bacterial specialities in the same way. Several properties are also unique among bacteria such as the murein wall polymer, endospores, photosynthesis, and Calvin cycle for CO_2 fixation. These features are not found among archaea. In Table 4 an attempt is made to make a list of thermophilic bacteria as was done in Table 3 for archaea. It is interesting to note that there are many more anaerobes than aerobes among thermophilic archaea, but about equal numbers of aerobes and anaerobes are known among thermophilic bacteria. This observation can probably be rationalized because of the low solubility of oxygen at the high temperatures where archaea dominate. For further information on the properties of the individual bacterial groups, the reader is referred to other chapters in this book.

IV. ECOLOGY OF THERMOPHILES

A. GEOTHERMAL HABITATS

Geothermal activity is mainly connected with tectonic activity where the earth's plates are moving, either drifting away from each other or colliding. These areas are characterized by volcanic activity and in many places geothermal areas on the surface. Geothermal areas are mainly of two types: one type is solfatara fields with much sulfur, acidic soils, acidic hot springs, and boiling mud pots; the other type is characterized with fresh-water hot springs and geysers and neutral-to-alkaline pH.[1,99,100]

Natural geothermal areas are found in all parts of the globe associated with tectonic activity, but usually concentrated in small places. The best known geothermal areas and most studied biologically are in Iceland, North America (Yellowstone National Park), New Zealand, Japan, Italy, and the U.S.S.R. 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; it is considered as one of the earth's *hot spots* because of the amount of geothermal energy which comes to the surface there. Geothermal areas cover in total over 500 km^2 which is about 0.5% of the total surface of the country.

Geothermal areas can generally be divided into two classes according to the heat source. One type is the so-called *high temperature fields* located within the active volcanic zones and having a magma chamber at a depth of 2 to 5 km as a heat source. It is usually characterized by water temperatures of 150 to 350°C at a depth of 500 to 3000 m and by emissions of steam and volcanic gases on the surface.[101] 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. Also traces of methane, ammonia, and carbon monoxide are often found.[102] 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.[1] 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 to 2.5 where sulfuric acid (pK_2 = 1.92) is the effective buffering agent.[99] Because of the high temperature, there is little water which comes to the surface and the hot springs are usually in the form of 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 soils in such solfatara fields are not nearly as acidic as the hot springs themselves. The soils are usually very dense and moist and are reduced by high sulfide. Only the top 1 to 2 cm are oxidized and acidic but below the pH maybe only 5 to 6.[2]

The other type of geothermal areas are so-called *low temperature fields* located outside the volcanically active zones, heated by deep lava flows or by dead magma chambers. The water temperature at a depth of 500 to 3000 m is usually below 150°C.[103] 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 here the subsurface pH is near neutrality, but there is usually much more water and little sulfide so that its surface oxidation has no effect on the pH. On the surface, however, the CO_2 is blown away and the silica precipitates, which results in increased pH. The pH is often stabilized at 9 to 10 where silicate (pK_n = 9.7) and carbonate (pK_2 = 10.25) start to act as the effective buffering agents.[99] Since these areas are located outside the volcanically active zone, they are geologically rather stable and the individual hot springs are very constant both in temperature and waterflow. However, they are often disrupted or new ones created during earthquake activities.

Both types of geothermal fields can also be found on the seafloor, adding salinity to the other factors.[2] Also here the sulfide gets oxidized to sulfur and sulfuric acid but there is so much water around that it cannot affect the pH to the same extent as in the terrestrial fields. Microniches with low pH might, however, still be formed.

B. OTHER THERMAL HABITATS

Constant natural hot habitats other than geothermal are very few, with burning coals perhaps the only one.[1] 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 can spontaneously ignite. These are, however, very transient ecosystems and there are mainly rapidly growing spore formers which can take advantage of such biotopes. Several man-made, constant hot environments have been created. These include hot water pipelines and heat exchangers in homes and factories, burning coal refuse piles, and thermophilic waste treatment plants.[6,104-107] Numerous processes in the food and chemical industries use evaporation or extraction, which is run at high temperatures and which often creates ideal conditions for the growth of thermophilic microorganisms.[6,106] Several well-known thermophiles have been isolated from such man-made systems, and some have not even been found elsewhere.[79,98,107]

C. ECOLOGY OF THERMOPHILIC ARCHAEA

All known methanogens are neutrophilic, strictly anaerobic archaea, and it seems that they can be isolated from all anaerobic habitats, at low or high temperature. They grow mainly by oxidizing hydrogen with CO_2 as the electron acceptor and forming methane, or on acetate and a few other simple compounds containing methyl groups.[30,41] A few of the thermophilic methanogens have been isolated from sewage treatment systems but most have been isolated only from hot springs.[2,108] The hyperthermophilic methanogens such as *Methanothermus fervidus* are neutrophilic autotrophs growing on hydrogen and CO_2.[24] They have been isolated from hot springs with a pH at around 6.5 and from hot soils at a depth of 30 cm where the pH was about 6.0, although the pH was very acidic on the surface.[2,42] These environments are very reduced with high sulfide concentrations.

All members of the sulfur-utilizing archaea, with the exception of the moderate thermophile *Thermoplasma acidophilum*, have been isolated only from geothermal areas.[2,46] The aerobic genera *Sulfolobus* and *Acidianus* are also acidophilic growing optimally at pH 2 to 3.[2,25,26] This is, of course, in accordance with their metabolism of oxidizing sulfur to sulfuric acid and their natural habitats of hot acidic springs and soils. *Thermoplasma* was first isolated only from burning coal refuse piles in N. America but recently also has been isolated from geothermal fields.[34,46]

The sulfur-metabolizing anaerobes, on the other hand, are moderately acidophilic or

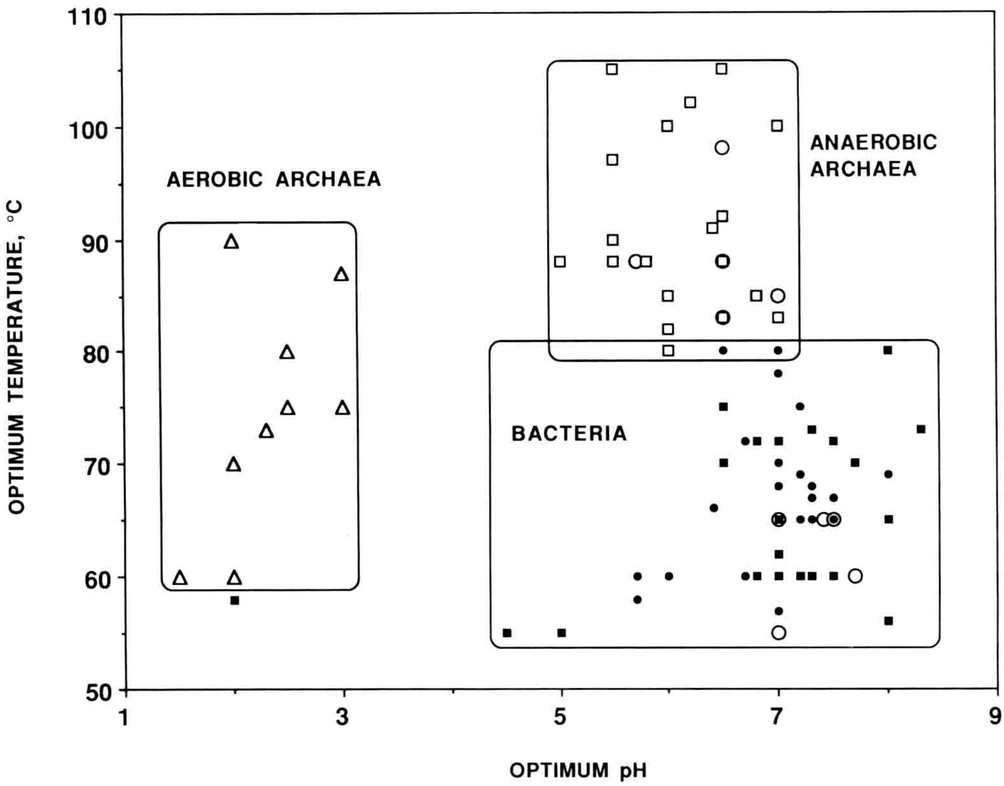

FIGURE 1. The distribution profile of thermophilic procaryotes according to T_{opt} and pH_{opt}. Archaea are marked with open symbols and bacteria with closed symbols. The symbols are ○, methanogens; △, aerobic archaea; □, anerobic archaea; ■, aerobic bacteria; ●, anaerobic bacteria.

neutrophilic, most growing optimally at pH 5.5 and some even up to pH 7.0.[2] Since they are either obligate or facultative sulfur-reducing organisms, they must grow close to the surface, where elemental sulfur accumulates from the oxidation of H_2S. On the oxygen side of the sulfur layer it is further oxidized to sulfuric acid which will partly diffuse into the anaerobic part, making the organisms' environment mildly acidic. This is clearly reflected in the optimum pH of 5 to 6 for most of the anaerobic thermophilic archaea (Figure 1). It can be concluded that thermophilic archaea are really much more extremophilic than bacteria, especially in terms of acidity and temperature.

D. ECOLOGY OF THERMOPHILIC BACTERIA

The ecology of thermophilic bacteria is, in many respects, more complicated than that of thermophilic archaea since there is more diversity both in respect to metabolism and habitats, but at lower temperature. Aerobic spore-forming bacilli can grow from pH 2 to 9 (see Chapter 2). They easily can be isolated from hot springs, but generally also from a number of other habitats that are only heated transiently and from permanently cold places. Some are facultative anaerobes and can either ferment or use nitrate as an electron acceptor under anaerobic conditions.[68,109]

Neutral and alkaline hot springs are the main habitat of nonspore-forming aerobes although some have also been isolated from hot pipes or factories.[104-107,110] They are obligate aerobes except for some that can grow anaerobically with nitrate as an electron acceptor, forming either nitrite or nitrogen gas. They grow mostly at neutral or alkaline pH, and none are really acidophilic. Organisms in this group are normally not isolated from acidic solfatara