

# Chapter 1

## Diversity of Hot Environments and Thermophilic Microbes

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**Abstract** The existence of life at high temperatures is quite fascinating. At both ends of the temperature range compatible with life, only microorganisms are capable of growth and survival. A great variety of microbes survives and grows at such elevated temperatures. Many thermophilic microbial genera have been isolated from man-made (acid mine effluents, biological wastes and waste treatment plants, and self-heated compost piles) and natural (volcanic areas, geothermal areas, terrestrial fumaroles, terrestrial hot springs, deep-sea hydrothermal vents, geothermally heated oil and petroleum reserves, sun-heated soils/sediments) thermal habitats throughout the world. Both culture-dependent and culture-independent approaches have been employed for understanding the diversity of microbes in hot environments. These organisms not only tolerate such high temperatures but also usually require these for their growth and survival and are known as thermophiles/thermophilic microbes, which include a wide variety of prokaryotes (bacteria and archaea) as well as eukaryotes. Interest in their diversity, ecology, and physiology and biochemistry has increased enormously during the past few decades. These organisms have evolved several structural and chemical adaptations that allow them to survive and grow at elevated temperatures.

**Keywords** Thermophile • Natural hot habitats • Man-made hot habitats • Hot water springs • Deep-sea hydrothermal vents • Acid mine drainage

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## 1.1 Introduction

Of enormous range of temperatures known (from 0 to approx.  $3 \times 10^9$  K), only a minute fraction is compatible with life. It is now well known that microbial life can prevail or thrive in the upper as well as lower temperature limits that are known to be compatible with life. Temperature creates a series of challenges, from structural devastation due to ice crystal formation at one extreme to the denaturation of biomolecules and cell components at the other. A wide variety of microorganisms have, however, been discovered that can overcome these challenges. One such group of microorganisms includes thermophiles. The word “thermophile” has been derived from two Greek words “thermotita” (meaning heat) and “philia” (meaning love). Thermophiles are heat-loving organisms, which not only tolerate high temperatures but also usually require these for their growth and survival. A thermophile as defined by Brock (1978) is “an organism capable of living at temperatures at or near the maximum for the taxonomic group of which it is a part.” Temperatures for their growth range from 50°C to as high as 121°C, the temperature used for sterilization in autoclaves. These microorganisms have been classified into moderate thermophiles, extreme thermophiles, and hyperthermophiles (Table 1.1).

The currently known upper temperature limit for life is 121°C (Kashefi and Lovley 2003) that corresponds to the maximum temperature limit for the growth and survival of nonphotosynthetic prokaryotes. The photosynthetic prokaryotes are unable to grow at such high temperatures or even closer and show the upper temperature limit of 70–73°C. Thermophilic prokaryotes have been known for over 90 years, while hyperthermophilic prokaryotes have been recognized only three decades ago (Brock 1978; Stetter et al. 1981; Zillig et al. 1981; Stetter 1982). The upper temperature limit for eukaryotic microorganisms is even lower, approximately 60–62°C (Tansey and Brock 1972), at which only a few species of fungi can grow. The upper temperature limits for eukaryotic algae and protozoa are slightly lower (55–60°C). Figure 1.1 presents temperature limits of growth for different taxonomic groups.

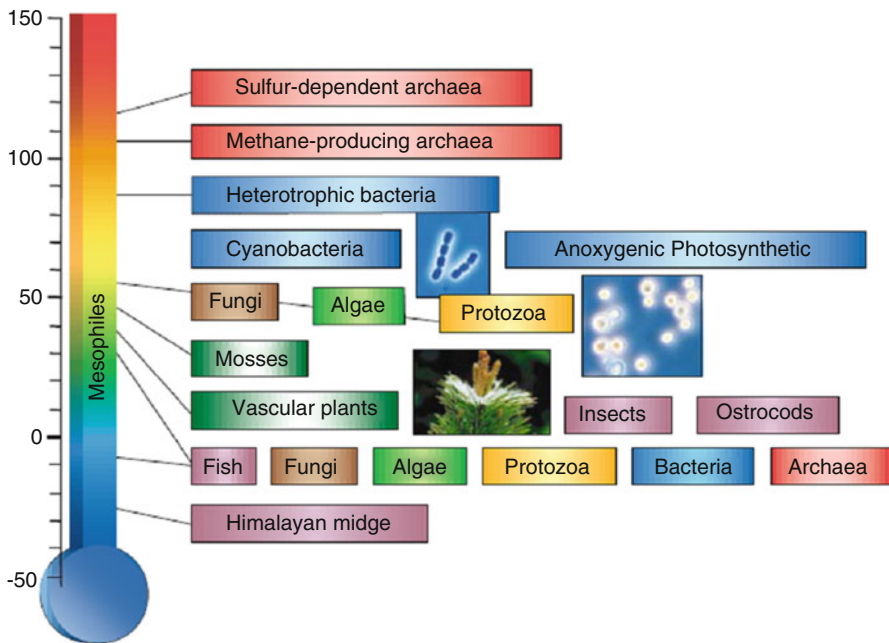
Within the past few decades, a great diversity of microorganisms has been discovered that exist in hot environments, both natural and man-made. This chapter deals with the diversity of thermophilic microorganisms and their environments and adaptations, which allow them to survive and grow at elevated temperatures. Although many thermophiles belonging to phylogenetically distinct groups have been cultured and are being used for various biotechnological applications, an unanticipated diversity of thermophilic species within high-temperature environments is evident by direct microscopic examination, 16S rDNA amplification, and other culture-independent techniques. This indicates that there are many more thermophiles that are yet to be cultivated.

## 1.2 Habitats with Elevated Temperatures

Habitats for the occurrence of thermophiles may be natural or man-made.

**Table 1.1** Classification of thermophilic microorganisms

Category	Temperature optima (°C)	Examples
Moderate thermophile	40–60°C	<i>Tepidibacter</i> , <i>Clostridium</i> , <i>Exiguobacterium</i> , <i>Caminibacter</i> , <i>Lebetimonas</i> , <i>Hydrogenimonas</i> , <i>Nautilia</i> , <i>Desulfonauticus</i> , <i>Sulfurivirga</i> , <i>Caminicella</i> , <i>Vulcanibacillus</i> , <i>Marinotoga</i> , <i>Caldithrix</i> , <i>Sulfobacillus</i> , <i>Acidimicrobium</i> , <i>Hydrogenobacter</i> , <i>Thermoplasma</i> , <i>Mahella</i> , <i>Thermoanaerobacter</i> , <i>Desulfovibrio</i>
Extreme thermophile	60–85°C	<i>Methanocaldococcus</i> , <i>Thermococcus</i> , <i>Palaeococcus</i> , <i>Methanotorris</i> , <i>Aeropyrum</i> , <i>Thermovibrio</i> , <i>Methanothermococcus</i> , <i>Thermosipho</i> , <i>Caloranaerobacter</i> , <i>Thermodesulfobacterium</i> , <i>Thermodesulfatator</i> , <i>Deferribacter</i> , <i>Thermosipho</i> , <i>Desulfurobacterium</i> , <i>Persephonella</i> , <i>Kosmotoga</i> , <i>Rhodothermus</i> , <i>Desulfurobacterium</i> , <i>Balnearium</i> , <i>Acidianus</i> , <i>Thermovibrio</i> , <i>Marinithermus</i> , <i>Oceanithermus</i> , <i>Petrotoga</i> , <i>Vulcanithermus</i> , <i>Carboxydobrachium</i> , <i>Thermaerobacter</i> , <i>Thermosulfidibacter</i> , <i>Metallosphaera</i>
Hyperthermophile	>85°C	<i>Geogemma</i> , <i>Archaeoglobus</i> , <i>Methanopyrus</i> , <i>Pyrococcus</i> , <i>Sulfolobus</i> , <i>Thermoproteus</i> , <i>Methanothermus</i> , <i>Acidianus</i> , <i>Ignisphaera</i> , <i>Ignicoccus</i> , <i>Geoglobus</i>



**Fig. 1.1** Temperature limits for different taxonomic groups (Rothschild and Mancinelli 2001)

### ***1.2.1 Natural Thermophilic Habitats***

Natural biotopes for the occurrence of thermophilic microorganisms are distributed worldwide. These are relatively high-temperature habitats, as compared to those which are anthropogenic or man-made. These may be terrestrial or marine in origin. The most remarkable ones that harbor a considerable variety of thermophilic microorganisms include terrestrial geothermal and volcanic areas and deep-sea hydrothermal vents (submarine hydrothermal vents). Most of the currently known extreme thermophiles and hyperthermophiles have been recovered from these regions by culture-dependent as well as culture-independent approaches. Geothermal and volcanic areas include terrestrial fumaroles (e.g., solfataras), terrestrial hot springs, and geysers. Others natural biotopes include geothermally heated oil and petroleum reservoirs and sun-heated soils/sediments.

#### **1.2.1.1 Geothermal and Volcanic Areas**

One of the most important natural biotopes of thermophiles includes geothermal and volcanic areas. These are worldwide in distribution and associated primarily with tectonically active zones where major crustal movements of the Earth occur. It is here in these areas where tectonic plates are highly converging or diverging. In the terrestrial zones, deep-seated magmatic materials are thrust close to the Earth's surface and serve as heat sources. As a result, groundwater percolating into the Earth becomes intensely heated but does not boil because of lithostatic pressure. When the percolating fluid reaches a sufficiently high temperature, the pressure generated forces the fluid through pores and fissures back to the surface of the Earth. These may lead to the formation of fumaroles, hot springs (or thermal springs), and geysers. These thermal habitats are concentrated in the small areas called thermal basins. In general, a thermal basin has geysers and/or flowing springs at the bottom of the hill, sulfur- and iron-rich non- (or weakly) flowing springs at the flanks of the hill, and fumaroles on the highest grounds. Highly mineralized geysers and geothermal waters at the bottom of a thermal basin have extensive water flow, neutral to alkaline pH, and constant temperature. Major components include bicarbonates and chlorides. The springs have progressively less flow and become less mineralized and more acidic because of the presence of sulfates (solfataras), as we move upslope. The temperatures are variable in these due to changes in steam sources beneath them.

#### **Terrestrial Fumaroles and Solfataras**

A fumarole (also known as steam vent) is an opening in the Earth's crust, often in the volcanic areas, which emits steam and gases such as carbon dioxide, sulfur dioxide, hydrochloric acid, and hydrogen sulfide. It has so little water that it all flashes into steam before reaching the surface. Fumaroles may occur along tiny cracks or

long fissures and on the surfaces of lava flows. Solfataras refers to fumaroles that emit sulfurous gases. Solfataras are found within the solfataric fields. Solfataric fields consist of soils, mud pots, and surface waters (sulfur-rich springs), heated by volcanic exhalations from the magma chambers located below them. They may have temperatures up to 100°C.

The chemical composition of solfataric fields is very variable and depends on the surrounding geology. Solfataras can be mildly acidic to slightly alkaline (pH 5–8) or extremely acidic, with pH values below 1.0. Acidic condition occurs due to the production of sulfuric acid ( $\text{H}_2\text{SO}_4$ ) from the biological oxidation of  $\text{H}_2\text{S}$  and  $\text{S}^\circ$ . Such hot and sulfur-rich environments are found throughout the world, including Italy, Iceland, New Zealand, and Yellowstone National Park in Wyoming (USA). Many of them are rich in iron minerals like ferric hydroxides and pyrite. Steam within the solfataric exhalations is mainly responsible for the heat transfer. In addition, they may contain  $\text{CO}_2$ ; variable amounts of  $\text{H}_2$ ,  $\text{CH}_4$ ,  $\text{N}_2$ , and  $\text{CO}$ ; and traces of nitrate and ammonia. Less common compounds like magnetite and the arsenic minerals may be present at some sites, for example, at Uzon caldera, Kamchatka, in Russia.

#### *Diversity of Thermophilic Microorganisms in Solfataric Fields*

Solfataric fields are colonized by a variety of acidophilic moderate and extreme thermophiles. Thermophilic members of bacteria and archaea have been recovered from solfataric habitats that include the species of *Pyrobaculum*, *Sulfolobus*, *Thermofilum*, *Methanothermus*, *Thermoproteus*, *Acidianus*, *Aciditerrimonas*, *Desulfotomaculum*, *Picrophilus*, *Alicyclobacillus*, *Thermoanaerobacter*, and *Thermogymnomonas*. *Thermoproteus neutrophilus*, *Thermoproteus tenax*, and *Pyrobaculum islandicum* are chemolithoautotrophs that reduce  $\text{S}^\circ$  by  $\text{H}_2$  anaerobically. While *Pyrobaculum aerophilum* grows anaerobically by the reduction of nitrate by  $\text{H}_2$  and on  $\text{H}_2$  and  $\text{O}_2$ , under microaerophilic conditions (Völkl et al. 1993). Strains of *Pyrobaculum organotrophum*, *Thermoproteus uzoniensis*, and *Thermofilum* are obligate heterotrophs growing on organic substrates by sulfur respiration. *Thermoproteus tenax* and *Pyrobaculum islandicum* are facultative heterotrophic sulfur respirers. Members of the genus *Sulfolobus* have been isolated from solfataric fields and acidophilic mud springs. *Sulfolobus acidocaldarius* has been isolated from Yellowstone National Park (the first hyperthermophilic microorganism isolated) (Brock et al. 1972) and *Sulfolobus solfataricus* strain P2, from a solfataric field near Naples, Pisciarelli, Italy (Zillig et al. 1980). *Sulfolobus* strains are hyperthermophilic crenarchaea that grow aerobically at optimum temperature and pH of 75–80°C and 2.5–3.5, respectively. *S. solfataricus* grows on a variety of different carbon sources like tryptone, various sugars, and amino acids. *Acidianus*, unlike *Sulfolobus*, can grow both aerobically as well as anaerobically. Under aerobic conditions, it oxidizes  $\text{S}^\circ$  to  $\text{H}_2\text{SO}_4$ , with  $\text{O}_2$  as electron acceptor. Anaerobically, it uses  $\text{S}^\circ$  as an electron acceptor,  $\text{H}_2$  as electron donor, and forming  $\text{H}_2\text{S}$  as the product (Seeger et al. 1986; Plumb et al. 2007). *Desulfotomaculum solfataricum*, isolated from hot solfataric fields of Iceland, uses sulfate, sulfite, or thiosulfate as electron

acceptors. The optimum temperature and pH for growth are 60°C and pH 7.3, respectively (Goorissen et al. 2003). The aerobic heterotrophs *Picrophilus oshimae* and *Picrophilus torridus*, isolated from Japanese soils permeated with solfataric gases, show optimal growth at pH 0.7 and 60°C (Schleper et al. 1995). The photosynthetic green alga, *Cyanidium*, that grows up to 55°C has been found to grow in the walls of the solfataric springs (Brock 1978). In Tengchong solfataric region, acidothermophilic sulfur oxidizers such as *Acidianus tengchongensis* and *Sulfolobus tengchongensis* have been found to play important roles in sulfur oxidation (He et al. 2004; Xiang et al. 2003). Most of the knowledge on the diversity studies of solfataras is based on cultivation studies. Using DNA-based culture-independent technique, up to 65 different phylotypes of crenarchaeotes have been described from one solfataras (Kvist et al. 2005; Siering et al. 2006). In most of the studies, many and often most sequences are only distantly related to cultivated relatives. In a study of a solfataras in Pisciarelli (Naples, Italy), 42% of the 201 analyzed clones have been found to cluster within the I.1b group crenarchaeota (Kvist et al. 2005). Kvist et al. (2007) analyzed genetic diversity of archaea by the analysis of amplified 16S rRNA genes and terminal restriction fragment length polymorphism (t-RFLP) from solfataras at the thermal active area of Hverageroi (Iceland). It has been reported that 18.0% of the total number of clones belonged to group I.1b Crenarchaeota, which was represented by only mesophilic species till now. Many other novel thermophilic isolates have been described by Plumb et al. (2007), Lee et al. (2007), and Itoh et al. (2007, 2011) from different solfataric fields. A list of thermophilic microorganisms isolated from solfataric fields is presented in Table 1.2.

### Terrestrial Hot Springs

A hot spring is a spring that is produced by the emergence of geothermally heated groundwater from the Earth's crust. Geothermal systems are present in many geological settings and not necessarily related to volcanism. The water arising from a hot spring is either heated by geothermal heat, that is, heat from the Earth's interior, in a nonvolcanic area, or by coming in contact with magma (molten rock). In the nonvolcanic area, groundwater that percolates deeply into the Earth's crust comes in contact with the rocks that get heated as a result of geothermal gradient. While in the volcanic areas that are the tectonically active zones, high-temperature gradient near magma may cause water to be heated enough that it becomes superheated. The pressure is generated that forces the water to the Earth's surface through pores and fissures within the Earth's crust, where it can issue as a hot spring (thermal springs) or geysers. As the hot fluid passes up through the Earth's surface, minerals dissolve from the adjoining areas, thus accounting for extensive mineralization. Hot-spring waters usually show high concentrations of many elements and can be highly supersaturated with a variety of minerals. These usually include H<sub>2</sub>S, CO<sub>2</sub>, low-molecular-weight organic compounds, CH<sub>4</sub>, H<sub>2</sub>, NH<sub>3</sub>, and trace elements (Brock 1978). The chemical interaction with the reservoir rocks and the rock forming minerals along

**Table 1.2** List of thermophiles isolated from solfataric fields

Genus/species	$T_{opt}$ (°C)	pH	Reference
<i>Sulfolobus acidocaldarius</i>	75	1–5	Brock et al. (1972)
<i>Thermoproteus tenax</i>	80	2.5–3.5	Zillig et al. (1980)
<i>Sulfolobus solfataricus</i>	88	2.5–6	Zillig et al. (1981)
<i>Desulfurococcus mobilis</i>	85	6	Zillig et al. (1982)
<i>Thermofilum pendens</i>	88	4–6.5	Zillig et al. (1983)
<i>Acidianus infernus</i>	88	1.5–5	Segerer et al. (1986)
<i>Thermoproteus neutrophilus</i>	88	6–7	Stetter (1986)
<i>Methanothermus sociabilis</i>	88	5.5–7.5	Lauerer et al. (1986)
<i>Acidianus ambivalens</i>	80	2.5	Segerer et al. (1986)
<i>Pyrobaculum islandicum</i>	100	5–7	Huber et al. (1987)
<i>Pyrobaculum organotrophum</i>	102	6	Huber et al. (1987)
<i>Pyrobaculum aerophilum</i>	100	5.8–9	Völkl et al. (1993)
<i>Picrophilus oshimae</i>	60	0.7	Schleper et al. (1995)
<i>Picrophilus torridus</i>	60	0.7	Schleper et al. (1995)
<i>Desulfotomaculum solfataricum</i>	60	7.3	Goorissen et al. (2003)
<i>Acidianus tengchongensis</i>	70	2.5	He et al. (2004)
<i>Acidianus sulfidivorans</i>	74	0.8–1.4	Plumb et al. (2007)
<i>Thermoanaerobacter sulfurigignens</i>	63–67	5–6.5	Lee et al. (2007)
<i>Thermogymnomonas acidicola</i>	60	1.8–4	Itoh et al. (2007)
<i>Aciditerrimonas ferrireducens</i>	50	2–4.5	Itoh et al. (2011)

the ascent path affects the composition of the hot water, which may be highly acidic or alkaline. Their temperature ranges from boiling or superheated at their sources to ambient temperature at their shores. Hot springs are present in many countries throughout the world. Countries that are renowned for their hot springs include Iceland, New Zealand, Chile, and Japan, but there are interesting and unique hot springs at many other places as well. The Yellowstone National Park (Wyoming, USA) has one of the highest numbers of hot springs in the world. Some examples of hot springs are presented in Tables 1.3 and 1.4.

Some examples of hot-spring sites in India are Ganeshpuri (Vajreshwari), Manikaran (Himachal Pradesh), Bendru Theertha (Karnataka), Chavalpani (Mahadeo Hills of Madhya Pradesh), Surya Kund (Bihar), Phurchachu (Reshi, Sikkim), Taptapani (near Berhampur), Atri (near Bhubaneswar), Tarabalo (Nayagarh District) of Orissa, Bakreshwar (West Bengal), and Tulsishyam (Gujarat).

A special type of hot spring is the geyser, where water and steam are ejected episodically through a vent. If the water is mixed with mud and clay, it is called a mud pot. Geysers exist in only a few places on Earth, so they are a fairly rare phenomenon. Generally all geyser field sites are located near active volcanic areas. Generally, surface water moves down to an average depth of around 2,000 m (6,600 ft) where it contacts hot rocks. The boiling of the pressurized water results in the geyser effect of hot water and steam spraying out of the geyser's surface vent. About a 1,000 known geysers exist worldwide, roughly half of which are in Yellowstone National Park, Wyoming, United States. Yellowstone Park is a lava plateau of an



**Table 1.3** List of novel thermophilic bacteria isolated from hot springs

Genus/species	Hot-spring location	T <sub>opt</sub> (°C)	Comments	References
<i>Aerobranca horikoshii</i>	Yellowstone National Park	57	Alkalitolerant	Engle et al. (1995)
<i>Thermotrix azorensis</i>	Sao Miguel Island	76–78	S <sup>o</sup> oxidizer	Odimtova et al. (1996)
<i>Meiothermus cerberus</i>	Iceland	55	–	Chung et al. (1997)
<i>Moorella glycerini</i>	Yellowstone National Park	58	Homoacetogen	Slobodkin et al. (1997)
<i>Porphyrobacter tepidarius</i>	Brackish hot spring	40–48	Photosynthetic	Slobodkin et al. (1997)
<i>Thermoterrabacterium ferritducens</i>	Yellowstone National Park	65	Iron reducer	Slobodkin et al. (1997)
<i>Streptomyces thermogriseus</i>	Yunnan	65–68	–	Xu et al. (1998)
<i>Caldicellulosiraptor kristjanssonii</i>	Iceland	78	Cellulolytic	Bredholt et al. (1999)
<i>Hydrogenobacter subterraneus</i>	Oita Prefecture, Japan	78	Heterotroph	Takai et al. (2001)
<i>Hydrogenophilus thermoluteolus</i>	Yellowstone National Park	63	H <sub>2</sub> oxidizer	Stohr et al. (2001)
<i>Thermoanaerobacter tengcongensis</i>	China	75	Anaerobe	Xue et al. (2001)
<i>Caloramator viterbensis</i>	Bagnaccio, Italy	58	Glycerol fermenter	Seyfried et al. (2002)
<i>Carboxydocella thermautotrophica</i>	Kamchatka Peninsula, Russia	58	CO utilizer	Sokolova et al. (2002)
<i>Meiothermus rosaceus</i>	Tengchong hot springs, Taiwan	55–60	Heterotroph	Chen et al. (2002)
<i>Pseudoxanthomonas broegbermensis</i>	Chi-ban hot springs, Taiwan	50–60	N <sub>2</sub> O producing	Chen et al. (2002)
<i>Roseiflexus castenholzii</i>	Japanese hot spring	50	Photosynthetic	Hanada et al. (2002)
<i>Microvirga subterranea</i>	Great Artesian Basin, Australia	41	–	Kanso and Patel (2003)
<i>Alicyclobacillus vulcanalis</i>	Coso hot springs, USA	55	Acidophile	Simbahan et al. (2004)
<i>Hydrogenivirga caldilitioris</i>	Kagoshima Prefecture, Japan	75	H <sub>2</sub> and S <sup>o</sup> oxidizer	Nakagawa et al. (2004)
<i>Phrylobacterium lituiforme</i>	Great Artesian Basin	40–41	Facultative anaerobe	Kanso and Patel (2004)
<i>Rubrobacter taiwanensis</i>	Lushan hot springs, Taiwan	60	Radiation resistant	Chen et al. (2004)
<i>Sulfurihydrogenibium azorensis</i>	São Miguel Island, Portugal	68	Microaerophile	Aguilar et al. (2004)
<i>Thermosinus carboxydivorans</i>	Yellowstone National Park	60	CO oxidizer	Sokolova et al. (2004)
<i>Lebetimonas acidiphila</i>	TOTO caldera, Mariana Arc	50	H <sub>2</sub> oxidizer	Takai et al. (2005)
<i>Methylothermus thermalis</i>	Japan	57–59	Methanotroph	Tsubota et al. (2005)
<i>Paenibacillus assamensis</i>	Assam, India	–	–	Saha et al. (2005)
<i>Silanimonas lenta</i>	Baekdu Mountain in Korea	47	Alkaliphile	Lee et al. (2005)
<i>Sulfurihydrogenibium yellowstonense</i>	Yellowstone National Park, USA	70	Facultative heterotroph	Nakagawa et al. (2005a)



<i>Thermincola carboxydiphila</i>	Lake Baikal region	55	Carboxydrotroph	Sokolova et al. (2005)
<i>Thiobacter subterraneus</i>	Hot spring at Hishikari mine, Japan	50–55	Chemolithoautotroph	Hirayama et al. (2005)
<i>Caldalkalibacillus thermarium</i>	China	60	Alkaliphile	Xue et al. (2006)
<i>Carboxydocella sporoproducens</i>	Karymsky Lake, Kamchatka, Russia	60	CO-utilizing anaerobe	Slepova et al. (2006)
<i>Cohnella laeviribosi</i>	Volcanic pond, Likupang	45	Ribose assimilator	Cho et al. (2007)
<i>Fervidobacterium changbaicum</i>	Changbai Mountains, China	75–80	Obligately aerobe	Cai et al. (2007)
<i>Geosporobacter subterraneus</i>	Paris Basin, France	42	Chemoorganotroph	Klouche et al. (2007)
<i>Planifilum yunnanense</i>	Yunnan, China	60	–	Zhang et al. (2007)
<i>Thermoanaerobacter pseudethanolicus</i>	Yellowstone National Park	–	Heterotrophic anaerobe	Onyenwoke et al. (2007)
<i>Ammonifex thiophilus</i>	Kamchatka, Russia	75	Chemolithotroph	Miroshnichenko et al. (2008b)
<i>Anoxybacillus bogrovensis</i>	Dolni Bogrov, Bulgaria	65	Alkalitolerant	Atanassova et al. (2008)
<i>Caldicellulosiruptor hydrothermalis</i>	Kamchatka, Russia	65	Cellulose degrader	Miroshnichenko et al. (2008a)
<i>Calditerrivibrio nitroreducens</i>	Yumata, Nagano, Japan	55	Nitrate reducer	Iino et al. (2008)
<i>Desulfotomaculum hydrothermale</i>	Tunisia	55	Sulfate reducer	Haouari et al. (2008a)
<i>Elitoraea tepidiphila</i>	São Miguel, Azores	45–50	Strict aerobe	Albuquerque et al. (2008)
<i>Sulfurihydrogenibium krisjanssonii</i>	Hveragerdi	68	H <sub>2</sub> and S° oxidizer	Flores et al. (2008)
<i>Sulfurihydrogenibium rodmanii</i>	Uzon caldera, Kamchatka	75	S° oxidizer	O'Neill et al. (2008)
<i>Thermoanaerobacter uzonensis</i>	Uzon caldera, Kamchatka, Russia	61	Anaerobic heterotroph	Wagner et al. (2008)
<i>Thermodesulfivibrio hydrogeniphilus</i>	Tunisian hot spring	65	Sulfate reducer	Haouari et al. (2008b)
<i>Thiofaba tepidiphila</i>	Fukushima Prefecture, Japan	45	S° oxidizer	Mori and Suzuki (2008)
<i>Venenivibrio stagnispumantis</i>	Watotapu, New Zealand	70	H <sub>2</sub> oxidizer	Hetzer et al. (2008)
<i>Caldanaerovirga acetigignens</i>	Black Rock Desert, NV, USA	62–66	Xylanolytic alkaliphile	Wagner et al. (2009)
<i>Carboxydotherrus siderophilus</i>	Kamchatka Peninsula, Russia	65	Hydrogenogenic	Slepova et al. (2009)
<i>Fervidicola ferrireducens</i>	Great Artesian Basin	70	Anaerobe	Ogg and Patel (2009a)
<i>Thermotalea metallivorans</i>	Great Artesian Basin, Australia	50	S° reducer	Ogg and Patel (2009b)
<i>Thermus islandicus</i>	Torfajokull, South Iceland	65	Mixotrophic S° oxidizer	Bjomsdottir et al. (2009)
<i>Anoxybacillus tengchongensis</i>	Tengchong and Eryuan, China	50	Alkalitolerant	Zhang et al. (2010, 2011)

(continued)

Table 1.3 (continued)

Genus/species	Hot-spring location	T <sub>opt</sub> (°C)	Comments	References
<i>Caldanaerobacter uzonensis</i>	Uzon caldera, Kamchatka, Russia	68–70	Heterotroph	Kozina et al. (2010)
<i>Caldilinea tarbellica</i>	France	55	Filamentous anaerobe	Grégoire et al. (2011)
<i>Thermus arcticformis</i>	Laitbin	70	Aerobe	Zhang et al. (2010, 2011)
<i>Chelatococcus sambhunathii</i>	–	42	Aerobe	Panday and Das (2010)
<i>Fervidicella metallireducens</i>	Great Artesian Basin, Australia	50	Anaerobe	Ogg and Patel (2010b)
<i>Hydrogenophilus islandicus</i>	Graendalur, Iceland	55	H <sub>2</sub> oxidizer	Vésteinsdóttir et al. (2011)
<i>Marinitoga litoralis</i>	Coastal spring, near Indian Ocean	60	Organotroph	Postec et al. (2010)
<i>Caloramator mitchellensis</i>	Great Artesian Basin, Australia	55	Anaerobe	Ogg and Patel (2011a)
<i>Ignavibacterium album</i>	Yumata, Nagano, Japan	45	Chemoheterotroph	Iino et al. (2010)
<i>Thermovenabulum gondwanense</i>	Great Artesian Basin, Australia	65	Fe-reducing anaerobe	Ogg and Patel (2010)
<i>Thiomonas islandica</i>	Graendalur, Iceland	45	H <sub>2</sub> and S <sup>0</sup> oxidizer	Vésteinsdóttir et al. (2011)
<i>Caloramator mitchellensis</i>	Great Artesian Basin, Australia	55	Anaerobic	Ogg and Patel (2011a)
<i>Hydrogenophilus islandicus</i>	Graendalur, Iceland	55	H <sub>2</sub> oxidizer	Vésteinsdóttir et al. (2011)
<i>Desulfosoma caldarium</i>	Colombian Andes, Colombia	57	Sulfate reducer	Baena et al. (2011)
<i>Jhaorihella thermophila</i>	Green Island (Lutao), Taiwan	55	–	Rekha et al. (2011)
<i>Fervidobacterium riparium</i>	Kunashir Island, Russia	65	Anaerobic, cellulolytic	Podosokorskaya et al. (2011)
<i>Schleiferia thermophila</i>	S o Miguel Island, Azores	50	–	Albuquerque et al. (2011)
<i>Meiothermus hypogaeus</i>	Himekawa hot spring, Japan	50	–	Mori et al. (2012)
<i>Laceyella sediminis</i>	Tengchong County, China	55	–	Chen et al. (2012)
<i>Brevibacillus aydinogluensis</i>	Karakoc hot spring, Turkey	50	–	Iino et al. (2010)
<i>Carboxydocella manganica</i>	Kamchatka hot spring	58–60	Mn(IV) and Fe(III) reducer	Slobodkin et al. (2012)
<i>Thermosyntropha tengcongensis</i>	Chinese hot spring	60	Degrades long-chain fatty acid	Zhang et al. (2012)

**Table 1.4** List of novel thermophilic archaea isolated from hot springs

Genus/species	Hot-spring location	T <sub>opt</sub> (°C)	Comments	References
<i>Sulfolobus hakonensis</i>	Hakone, Japan	70	Acidophile	Takayanagi et al. (1996)
<i>Sulfurisphaera ohwakuensis</i>	Ohwaku Valley, Japan	85	Acidophile	Kurosawa et al. (1998)
<i>Thermocladium modestes</i>	Japan	75	–	Itoh et al. (1998)
<i>Caldivirga maquilingensis</i>	Philippines	85	Sulfur reducer	Itoh et al. (1999)
<i>Sulfolobus yangmingensis</i>	Yangming National Park, Taiwan	80	Acidophile	Jan et al. (1999)
<i>Pyrobaculum oguniense</i>	Kumamoto Prefecture, Japan	90–94	Facultative aerobe	Sako et al. (2001)
<i>Caldisphaera lagunensis</i>	Philippines hot spring	70–75	Acidophile	Itoh et al. (2003)
<i>Caldisphaera lagunensis</i>	Philippines	70–75	Anaerobic heterotroph	Itoh et al. (2003)
<i>Desulfurococcus fermentans</i>	Uzon caldera, Kamchatka	80–82	Organoheterotroph	Perevalova et al. (2005)
<i>Ignisphaera aggregans</i>	New Zealand	92–95	Heterotroph	Niederberger et al. (2006)
<i>Acidobacillus saccharovorans</i>	Kamchatka hot spring, Russia	80–85	Acidophilic anaerobe	Prokofeva et al. (2009)
<i>Acidiplasma aeolicum</i>	Vulcano Island, Italy	45	Chemoorganotroph	Golyshina et al. (2009)
<i>Desulfurococcus kamchatkensis</i>	Uzon caldera, Kamchatka Peninsula, Russia	85	Protein degrading organotroph	Kublanov et al. (2009)
<i>Fervidococcus fontis</i>	Uzon caldera, Kamchatka Peninsula, Russia	65–70	Anaerobe	Perevalova et al. (2010)
<i>Methanothermobacter thermautotrophicus</i>	China	65	–	Ding et al. (2010)
<i>Metallosphaera cuprina</i>	Tengchong, Yunnan, China	65	Metal-mobilizing acidophile	Liu et al. (2011)

ancient date, in which the old centers of volcanic activity have been closed since a very long time but which still shows abundant hot-spring activity throughout its extent of nearly 60 miles north and south. Other major examples of geysers include the Valley of Geysers located in the Kamchatka Peninsula (Russia), El Tatio (Chile), Taupo Volcanic Zone (New Zealand), and Haukadalur (Iceland).

### *Microbial Mats*

Hot springs have become quite fascinating from a microbial ecologist's point of view as large areas are created with distinct thermal gradients as the water flows over the edges of the source and gradually cools as it descends. Thermal gradients that support microbial mat communities occur in various settings: between the center of geothermal hot springs and their edges, along the effluent channels that drain hot springs, as well as along the vertical near the ocean's surface (Brock 1978). The combination of continuous supply of nutrients (including high concentrations of inorganic reduced sulfur compounds) and a high light intensity over a relatively large area results in the formation of "microbial mats" representing tightly interacting communities of microorganisms. A wide diversity of thermophilic microorganisms exists in these mats (Ward et al. 1987). Thermophilic representatives of cyanobacteria, anoxygenic phototrophic bacteria, aerobic and anaerobic heterotrophs, methanogens, and sulfate reducers are all the components of these microbial mats.

The largest and best-studied hot-spring area is Yellowstone Park, Wyoming, USA. The upper temperature limit for phototrophic microbial mats found in neutral and alkaline hot springs in North America (Yellowstone National Park) is approximately 74°C (Castenholz 1984). A significant research attention has been focused on microbial mats that occur in hot-spring waters from 50 to 75°C. In general, microbial mats in this temperature range often consist of vertically organized communities in which the photosynthetic cyanobacterium *Synechococcus lividus* lives as a top layer, while the photosynthetic green non-sulfur-like bacterium *Chloroflexus aurantiacus* lives as an undermat. *Chloroflexus* utilizes bacteriochlorophylls *a* and *c*, chromatophores with a different absorption spectrum from the chlorophyll *a* (Chl *a*), characteristic of cyanobacteria, so sunlight at wavelengths poorly absorbed by the mat's top layers can be used. It has also been evidenced that significant symbiotic interactions may occur between these two organisms (Brock 1978; Mosser et al. 1974). Other species of algae, cyanobacteria, bacteria, archaea, and fungi also inhabit microbial mats in the hot springs at different locations, which primarily depends on their temperature variations and nutrient concentration.

Cyanobacterium *Synechococcus* occurs as a monospecific surface layer on "Synechococcus mats" (Ward et al. 1998). The phototrophic microbial mats of alkaline hot springs of Iceland are dominated by the cyanobacteria *Mastigocladus laminosus* or *Phormidium laminosum*, depending upon the temperature and sulfide concentration (Jørgensen and Nelson 1988). Furthermore, the mats contain large quantities of the green gliding phototrophic bacterium, *Chloroflexus aurantiacus*. Beneath the photosynthetic layer, anaerobic bacteria are present fermenting the

decaying mat. It has been observed that sulfate reduction is the main terminal pathway in high-sulfate (above 1 mM) hot springs, while methanogenesis is the main pathway in low-sulfate (below 1 mM) springs (Sandbeck and Ward 1981; Ward and Olson 1980). Nineteen genera and 36 species of microorganisms have been identified in various hot springs of Northern Thailand by morphometric analysis. The morphotypes, which dominated these hot springs in the temperature range of 40–80°C, are *Synechococcus lividus*, other *Synechococcus* sp., and *Phormidium boryanum* (Sompong et al. 2005). Iron-dominated microbial mats at chocolate pots hot springs (Yellowstone National Park) are composed of species of *Synechococcus*, *Oscillatoria*, *Chloroflexus*, *Pseudanabaena*, and *Mastigocladus* (Pierson and Parenteau 2000). Culture-dependent and culture-independent phylogenetic analysis of the freshwater thermal springs of Zerka Ma'in, Jordan, has been done. These hot springs are inhabited by a great diversity of thermophilic unicellular and filamentous cyanobacteria including *Thermosynechococcus*, *Chroogloeocystis*, *Fischerella* (*Mastigocladus*), and *Scytonema*. Based on 16S rRNA gene sequence analysis, it has been shown that the Zerka Ma'in strains are phylogenetically distinct from morphologically similar cyanobacteria found in hot springs worldwide (Oren et al. 2009). The isolation of a unicellular cyanobacterium with a 16S rRNA gene that showed 99% similarity with *Chroogloeocystis siderophila*, an organism originally found in iron-rich thermal environments in Yellowstone and requiring high iron concentrations for growth (Brown et al. 2005), is remarkable.

Three morphotypes of bacteria have been observed in sulfur-turf mats of Japan. Two of these have been tentatively named *Thiovibrio miyoshi* and *Thiothrix miyoshi*. Moreover, in situ ecophysiological and microscopic studies have shown that one of these bacteria, the large sausage-shaped "*Thiovibrio miyoshi*," predominates in sulfur-turf mats and oxidizes environmental sulfide to elemental sulfur and then to sulfate via thiosulfate (Maki 1991, 1993). In situ hybridization with clone-specific probes for 16S rRNA also revealed the presence of sausage-shaped bacteria in the hot-spring sulfur-turf mats in Japan (Yamamoto et al. 1998).

### *Diversity of Thermophilic Microbes in Hot Water Springs*

Hot springs also harbor other microorganisms other than those present in the microbial mats. Various culture-dependent and culture-independent studies have been carried out to understand their diversity in hot springs. It is generally accepted that less than 1% of the extant microbial "species" have been cultured (Ward 1998) and that the majority do not grow in conventional isolation media (Vetriani et al. 1998). Investigations of microbial diversity using culture-independent methods provide a valid approach to the assessment of true microbial diversity (Jeanthon 2000).

A number of extreme thermophiles or hyperthermophiles have been isolated from terrestrial hot springs (Ghosh et al. 2003; Marteinson et al. 2001; Takai and Sako 1999; Meyer-Dombard et al. 2005; Skirmisdottir et al. 2000; Tsubota et al. 2005; Miroshnichenko et al. 2008a, b; Wagner et al. 2008; Kublanov et al. 2009; Vésteinsdóttir et al. 2011). Phylogenetic characterization of microorganisms has been done for geothermal springs in different parts of world like Iceland (Skirmisdottir

et al. 2000; Marteinsson et al. 2001; Takacs et al. 2001), Yellowstone National Park (Hugenholtz et al. 1998; Ward et al. 1998), and Greece (Sievert et al. 2000). Phylogenetically unknown bacteria have been isolated from terrestrial hot springs (Aguilar et al. 2004; Sokolova et al. 2004; Lee et al. 2005; Nakagawa et al. 2005a; Tsubota et al. 2005; Slepova et al. 2006; Derekova et al. 2007). A great diversity of bacteria and archaea has been revealed by analysis of rRNA genes that were amplified by the polymerase chain reaction from environmental DNA (Barns et al. 1996; Burton and Norris 2000; Meyer-Dombard et al. 2005).

Hugenholtz et al. (1998) analyzed the SSU rRNA genes for studying the bacterial diversity of Obsidian Pool (OP), a Yellowstone National Park hot spring. Seventy percent of the sequence types have been found to be associated with 14 previously recognized bacterial phyla, and 30% of the sequence types were found to be unrelated with recognized bacterial divisions comprising 12 novel division-level lineages. Some of these sequences are nearly identical to those of cultivated chemolithotrophic thermophiles, including *Calderobacterium*, *Thermodesulfobacterium*, some Aquificales, and  $\delta$ -Proteobacteria. Similarly, a 16S rRNA-based study to reveal thermophilic bacterial communities in Indonesia's thermal springs reported 22 sequences assignable to the taxa of Proteobacteria, *Bacillus*, and *Flavobacterium* (Baker et al. 2001).

Thermophilic ammonia-oxidizing bacteria capable of growth at 55°C have been enriched from geothermal springs of Kamchatka (Golovacheva 1976). A thermophilic nitrate-reducing bacterium isolated from Garga spring has been identified as *Geobacillus gargensis* sp. nov. (Nazina et al. 2004). Culture-independent 16S rRNA-based techniques have revealed the presence of *Nitrospira* relatives in subterranean hot springs of Iceland (Marteinsson et al. 2001) and in a radon-containing hot spring in South Australia (Anitori et al. 2002). The presence of nitrifying bacteria in Garga hot spring at 45–59°C has also been described. They are identified as members of *Nitrosomonas* by both conventional and molecular techniques (DGGE analysis of partial 16S rRNA sequences). Nitrite-oxidizing bacteria have been identified as the members of *Nitrospira* (Lebedeva et al. 2005). A novel aerobic, obligately mixotrophic, moderately thermophilic, thiosulfate-oxidizing bacterium *Thiomonas bhubaneswarensis* sp. nov. has been isolated from hot-spring sediment samples collected from Atri, Bhubaneswar, India (Panda et al. 2009). Members of the genus *Geobacillus* have been isolated from the volcanic hot springs. For example, various strains of *Geobacillus thermoleovorans* have been isolated from the volcanic hot springs of Waimangu Volcanic Valley, New Zealand (Malhotra et al. 2000; Noorwez et al. 2006).

Culture-independent approach has also been used to study bacterial diversity of hot spring in Bakreshwar, India. 16S ribosomal DNA clones derived from the sediment samples are found to be associated with  $\delta$ -Proteobacteria (*Shewanella*), cyanobacteria (*Synechococcus elongates*), and green non-sulfur (*Thermus thermophilus*) and low-GC gram-positive bacteria (*Desulfotomaculum luciae*) (Ghosh et al. 2003). A novel species, *Anaerobranca californiensis*, has been isolated from the thermophilic alkaline hypersaline Mono Lake's sediment, California (Gorlenko et al. 2004).

A novel bacterium that transforms light into chemical energy has been discovered from Yellowstone National Park (Bryant et al. 2007). This organism has been identified by the 16S rRNA analysis and further verified by enrichment techniques and biochemical methods. This novel genus and species *Chloracidobacterium thermophilum* belongs to a new phylum, Acidobacteria. This is the third time in the past 100 years that a new bacterial phylum has been added to the list of those with chlorophyll-producing members. *Chloracidobacterium thermophilum* grows near the surface of the mats together with cyanobacteria, where there is light and oxygen, at a temperature of about 50–66°C. The organism has been found in three hot springs: Mushroom Spring, Octopus Spring, and Green Finger Pool in Yellowstone National Park. It has been found that *C. thermophilum* makes two types of chlorophyll (a and c) that allow these bacteria to thrive in microbial mats and to compete for light with cyanobacteria (Bryant et al. 2007).

Various thermophilic representatives of *Geobacillus*, *Anoxybacillus*, and *Bacillus* have been identified and characterized from diverse hot springs in Turkey by using phenotypic and genotypic methods including fatty acid methyl ester and rep-PCR profilings and 16S rRNA sequencing (Adiguzel et al. 2009). Another hot spring that has been studied is Kamchatka at Russia. Many thermophiles have been isolated from this hot spring. *Thermoproteus uzoniensis* (Bonch-Osmolovskaya et al. 1990), *Carboxydocella thermautotrophica* (Sokolova et al. 2002), *Anoxybacillus voinovskiensis* (Yumoto et al. 2004), *Carboxydocella sporoproducens* (Slepova et al. 2006), *Ammonifex thiophilus* (Miroshnichenko et al. 2008b), *Sulfurihydrogenibium rodmanii* (O'Neill et al. 2008), *Thermoanaerobacter uzonensis* (Wagner et al. 2008), and *Caldanaerobacter uzonensis* (Kozina et al. 2010) are some of the examples.

Recently whole genome of a bacterium *Acidilobus saccharovorans*, isolated from an acidic hot spring of Uzon caldera, Kamchatka (Russia), has been sequenced (Mardanov et al. 2010).

It is an obligately anaerobic acidophile with growth optimum at pH 3.5–4 and a temperature optimum of 80–85°C. It utilizes a wide range of proteinaceous and carbohydrate substrates and cannot grow lithoautotrophically on H<sub>2</sub> and CO<sub>2</sub> (Prokofeva et al. 2009). Thermophilic magnetotactic bacteria have been detected by 16S rRNA sequencing in the microbial mats of hot springs near Sierra Nevada Mountains in central California, where temperature up to 58°C has been found (Nash 2008). Population of a moderately thermophilic magnetotactic bacterium has also been discovered in Great Boiling Springs, Nevada. Amplified 16S rDNA has been obtained and sequenced, which was further authenticated by fluorescent in situ hybridization (FISH). A novel isolate *Candidatus Thermomagnetovibrio paiutensis* has been classified under the phylum Nitrospirae (Lefèvre et al. 2010).

In addition, there have been many studies on archaeal diversity in hot springs. PCR-mediated small-subunit rRNA gene (SSU rDNA) sequencing has been done to study archaeal diversity at hot-spring pools at Mt. Unzen, in Nagasaki Prefecture, Japan. This acidic hot spring which could be one of the most extreme habitats for life due to its high temperature (85–93°C) and strong acidity (pH 2.8) harbored archaeal species in majority, including thermoacidophilic *Sulfolobus* species and unidentified crenarchaeotal and korarchaeotal species (Takai and Sako 1999).



In an SSU 16S rDNA-based study of prokaryotic diversity of Bor Khlueng hot spring (Thailand), diversity of bacteria spanning 11 major lineages has been obtained. Almost 23% of the clones are classified as Acidobacteria. The other clones are grouped into the Nitrospirae, Proteobacteria, *Deinococcus–Thermus* lineage, Planctomycetes, and Verrucomicrobia. Archaeal (crenarchaeotal and korarchaeotal) clones were also obtained (Kanokratana et al. 2004).

Microbial community structure of Obsidian Pool, Sylvan Spring, and Bison Pool, Yellowstone National Park (Wyoming, USA), has been investigated by polymerase chain reaction (PCR) amplification of 16S rRNA gene sequences using environmental DNA. Diverse crenarchaea has been found to exist in all three pools, particularly affiliating with deep branching, but uncultivated organisms. Cloned DNA affiliating with the Desulfurococcales and Thermoproteales has also been identified (Meyer-Dombard et al. 2005).

A combination of both culture and culture-independent techniques revealed the presence of *Pyrobaculum* sp. and *Ignisphaera aggregans* in the near-neutral high-temperature hot spring in Rotorua, New Zealand (Niederberger et al. 2008).

Culture-independent (PCR with Crenarchaeota-specific primers and DGGE) and culture-dependent approaches have been used to study the diversity of Crenarchaeota in terrestrial hot springs of the Kamchatka Peninsula and the Lake Baikal region (Russia) and of Iceland. Both cultured (mainly hyperthermophilic) and uncultured Crenarchaeota have been obtained (Perevalova et al. 2008). Kozubal et al. (2008) isolated and characterized a thermophilic Fe-oxidizing thermophilic Crenarchaeum, *Metallosphaera*-like strain MK-1, from geothermal springs of Yellowstone National Park. This strain is shown to be a member of the order Sulfolobales, showing 94.9–96.1% sequence similarity to other known *Metallosphaera* spp. and less than 89.1% similarity to the known *Sulfolobus* spp.

Malkawi et al. (2010) investigated five major hot springs in Jordan (Ashounah, Waggas, Zara, Zarqa Ma'in, and Afra springs) using both culture-based and culture-independent approaches and obtained amplification of specific 16S rDNA sequences of bacteria, archaea, green sulfur bacteria, green non-sulfur bacteria, heliobacteria, and methanogenic archaea from metagenomic DNA extracted directly from water and mat samples from each thermal spring. Other examples of novel bacteria and archaea that have been recovered from different hot springs all over the world are presented in Tables 1.3 and 1.4.

There have been relatively few studies on isolation of fungi from hot springs. Five species of thermophilic and thermotolerant fungi have been isolated from hot springs of Northern Taiwan, which were identified as *Aspergillus fumigatus*, *Thermomyces lanuginosus* (syn. *Humicola lanuginosa*), *Humicola insolens*, *Penicillium duponti*, and *Rhizoctonia*. All these isolates can grow at temperatures ranging from 55 to 64°C (Chen et al. 2000). Several fungi have been isolated from near-neutral and alkaline thermal springs in Tengchong Rehai National Park. Internal transcribed spacer (ITS) sequencing combined with morphological analysis identified these fungi to the species level. These fungi are *Rhizomucor miehei*, *Chaetomium* sp., *Talaromyces thermophilus*, *Talaromyces byssochlamydoides*,

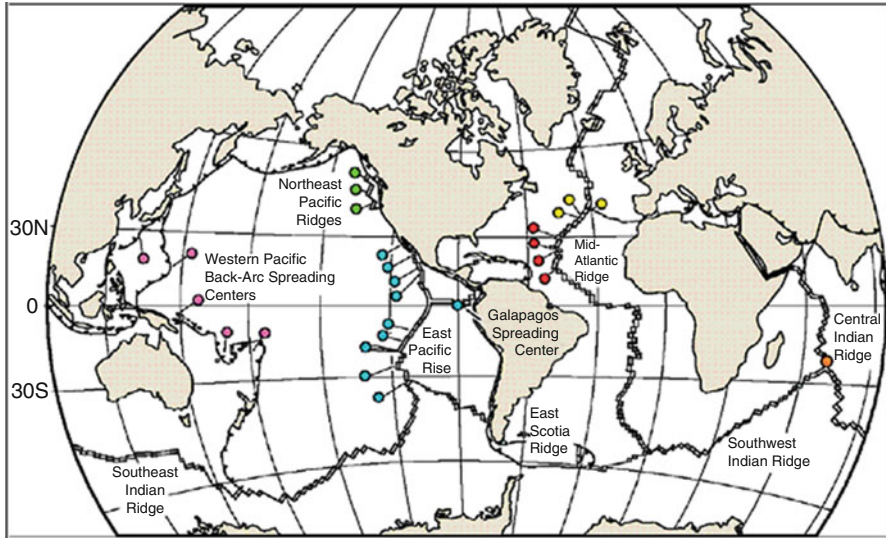
*Thermoascus aurantiacus* Miele var. *levisporus*, *Thermomyces lanuginosus*, *Scytalidium thermophilum*, *Malbranchea flava*, *Myceliophthora* sp., and *Coprinopsis* sp. Two species, *T. lanuginosus* and *S. thermophilum*, have been found to be the dominant species (Pan et al. 2010).

### 1.2.1.2 Deep-Sea Hydrothermal Vents

Microbial life was earlier thought to be restricted to the thin surface layer of the planet, where the organic matter derived from photosynthesis is present and is available as an energy and nutrient source and where temperatures and chemical conditions are conducive to the known living organisms (Jørgensen and Boetius 2007), and the deep sea, which represents 88% of the Earth's area covered by seawater and 75% of the total volume of the oceans (Prieur et al. 1995), was perceived as a cold, dark, high-pressure, and nutrient-poor environment inhabited by psychrophilic, oligotrophic, and barophilic microorganisms (Prieur 1997). The discovery of deep-sea hydrothermal vents in the Galapagos Rift in 1977 made a powerful impact on the development of deep-sea biology and microbiology of hydrothermal systems (Lutz et al. 1994; Taylor et al. 1999). For the first time, an ecosystem totally based on primary production achieved by chemosynthetic bacteria was discovered.

A hydrothermal vent is a fissure in the planet's surface from which geothermally heated water emerges. Hydrothermal vents are commonly found near those areas where tectonic plates are moving apart, like volcanic areas. Hydrothermal vents are found either on land or under the deep sea. Common hydrothermal vents found on land types include hot springs, fumaroles, and geysers. Hydrothermal vents under the sea are called deep-sea hydrothermal vents (commonly known as black smokers). Hydrothermal vents occur at depths of 800–3500 m, which corresponds to hydrostatic pressures of 80–350 bars (8–35 MPa) (Miroshnichenko 2004). At deep-sea hydrothermal vents, warm and/or hot fluids are emitted. Islands of highly dense and biologically diverse prokaryotic and eukaryotic communities exist in the immediate vicinity of hydrothermal vent, as compared to the surrounding bare seafloor. These communities comprise organisms with distinct metabolism based on chemosynthesis and growth rates comparable to those from shallow-water tropical environments (Thornburg et al. 2010).

Currently, over 300 deep-sea hydrothermal vent sites are known throughout the world (Desbruyères et al. 2006). These vent sites generally occur along a nearly continuous underwater mountain chain (mid-ocean ridges) comprising of more than 75,000 km that remains largely unexplored for hydrothermal activity (Van Dover 2000). Figure 1.2 shows major mid-ocean ridges and deep-sea hydrothermal vent sites throughout the world. These mid-ocean ridges, which are located at the boundaries between the tectonic plates of Earth's crust, are the sites of incremental seafloor spreading (spreading centers) at which molten rock (magma) rises toward Earth's surface as the tectonic plates move in relation to each other.



**Fig. 1.2** Map showing the major midocean ridges and known deep-sea hydrothermal vent biogeographic provinces: *pink*, Western Pacific; *green*, northeast Pacific; *blue*, East Pacific Rise; *yellow*, Azores; *red*, Mid-Atlantic Ridge; *orange*, Indian Ocean (Thornburg et al. 2010)

### Formation of Hydrothermal Vents

In the regions of tectonic activity, seawater interacts with the hot basaltic rocks below the ocean floor. As a result of this interaction, the water changes its chemical composition and gets overheated but still remains liquid because of the enormous hydrostatic pressure. This is called hydrothermal fluid (Miroshnichenko 2004). Hydrothermal fluids exiting vents are at the temperatures up to 400°C and are enriched with transition metals (e.g., aluminum, copper, cobalt, iron, lead, manganese, and zinc), silica, sulfides, and dissolved gases such as hydrogen and methane (Van Dover 2000; Seyfried and Mottl 1995).

The rapid mixing of these hydrothermal fluids with the surrounding cold seawater as they exit from the ocean floor causes changes in pH and temperature and the precipitation of metal sulfides and minerals (black smoke) to form columnar sulfide-rich chimney structures called black smokers. Depending on the amount of mixing with cold oceanic water before emission, hydrothermal fluids may have different physicochemical compositions (Priour 1997). Within a year of a volcanic eruption, mature black-smoker sulfide chimneys grow 10–20 m high and may have several high-temperature orifices near the top (Tivey 1991; Haymon 1983). White smokers are the chimney structures that are formed around intermediate temperature flows (100–300°C) and formed due to precipitation of silica, anhydrite, and barite as white particles. In addition, there are several other structural variations of sulfide-rich mineral deposits, including beehives (with horizontal layering and conduits for diffuse fluid flow), flanges (where pooled hot fluids are trapped beneath a shelf-like structure), and complex sulfide mounds (Van Dover 2000).

When the hot electron-donor-rich and anoxic vent fluids meet cold electron-acceptor-rich and oxygenic ocean waters, a temperature gradient is formed, and therefore, a variety of ecological niches form in these regions characterized by different temperatures, nature of substrate, and availability of electron donors and acceptors for energy metabolism. In these regions, the chemical energy becomes available to free-living chemolithoautotrophs microorganisms and those associated with animals as symbionts. Owing to the extreme gradients and diversity in physical and chemical factors, hydrothermal vents remain incredibly fascinating to microbiologists, and most studies on deep-water microbial communities are now focused on vent habitats.

### Diversity of Thermophilic Microorganisms at Deep-Sea Hydrothermal Vents

Hydrothermal vents have been one of the most fascinating environments for the search of extremophiles because of presence of high temperatures, hydrostatic pressures, reducing power, toxic chemistry, extreme pH, and fluctuations of environmental conditions (Takai et al. 2006b). In hydrothermal vents, microorganisms exist in either free-living form or remain attached to rocks, to sediment, or on/in vent animals, which in turn may feed directly on the microbes or engage in symbiotic associations to acquire fixed carbon and nitrogen (Tivey 1991; Haymon 1983; Robigou et al. 1993). Gradients of temperature and chemistry in hydrothermal vents support free-living microorganisms with a diverse physiologies and tolerances. These microorganisms can be detected hundreds of kilometers away from vent fields (Jannasch and Wirsén 1979; Juniper et al. 1988). They also form microbial mats of various colors and morphologies on the surface of basalt (Moyer et al. 1994; Santelli et al. 2008) and chimney spires (Kormas et al. 2006) and within hydrothermal sediments (Lopez-Garcia et al. 2003) where they serve as food for numerous invertebrate species (Van Dover and Fry 1994).

In the absence of light and presence of hydrothermal fluids rich in minerals, reduced compounds (including  $\text{H}_2\text{S}$ ,  $\text{CH}_4$ ), and  $\text{CO}_2$ , chemical energy replaces solar energy as the fuel that supports primary production by chemosynthetic bacteria and archaea (Kelley et al. 2002). Both lithotrophic and organotrophic isolates have been isolated from deep-sea vents. The majority of the recent isolates are lithotrophs, obligately or facultatively dependent on inorganic energy sources, and obligate or facultative anaerobes. This is in good agreement with the environmental conditions that exist within hydrothermal fluid. Owing to high concentration of  $\text{H}_2\text{S}$  within hydrothermal fluids (3–110 mmol per kg seawater), sulfide oxidation is a dominant microbial source of energy in vent communities (Kelley et al. 2002). The aerobes use  $\text{O}_2$  as the electron acceptor during the energy-yielding chemosynthetic reaction, while anaerobes use  $\text{CO}_2$ ,  $\text{Fe}^{3+}$ ,  $\text{NO}_3^{2-}$ , or organic compounds (to oxidize  $\text{H}_2$ ) (Kelley et al. 2002).

The diversity of hydrothermal vent microbial communities cannot truly be determined by artificial culture-dependent techniques, since 99% of marine microbes are unculturable (Amann et al. 1995). Thus, molecular phylogeny-based approach has been used using nucleotide-sequence analysis of the highly conserved gene for the

small-subunit (SSU) rRNA molecule (16S rRNA) (Pace 1997). New phylotypes, often representing major new lineages, have been shown consistently with each molecular analysis of microbial environments (Pace 1997; Venter et al. 2004; Sogin et al. 2006). For example, Huber et al. (2002a) identified a new archaeal phylum known as “Nanoarchaeota” by the analysis of PCR-amplified SSU rRNA genes from a defined coculture of hyperthermophilic archaea. Similar methods have indicated the emergence of a newly defined lineage distributed throughout the global deep-sea vent system called as the “deep-sea hydrothermal vent Euryarchaeota group” (DHVEG) (Takai and Horikoshi 1999).

Microbial communities of deep-sea hydrothermal vents include microorganisms of both the bacteria and archaea domains. In the last decades of the twentieth century, significant work on the diversity of thermophiles inhabiting hydrothermal environment has been done. At that time, most of known deep-sea thermophilic prokaryotes were represented by hyperthermophilic archaea (Blöchl et al. 1995; Prieur et al. 1995). The thermophilic archaeal lithotrophs included *Methanococcus* (Jones et al. 1983; Zhao et al. 1988; Jeanthon et al. 1999) and the endemic genus *Methanopyrus* (Kurr et al. 1991), characterized by temperature optimum at 98°C and maximum at 110°C, a hydrogen-oxidizing *Pyrolobus fumarii* (Blöchl et al. 1997) that could grow at even higher temperatures (optimum at 106°C and maximum at 113°C) by means of nitrate-, thiosulfate-, or microaerobic respiration and sulfate- and thiosulfate-reducing archaea *Archaeoglobus* (Burggraf et al. 1990; Huber et al. 1997). Organotrophic thermophilic archaea were represented by numerous obligately anaerobic members of Thermococcales (Zillig and Reysenbach 2001) and various species of the genera *Pyrodictium* (Pley et al. 1991) and *Staphylothermus* (Fiala et al. 1986).

At that time, thermophilic bacteria attracted much less attention of microbiologists than archaea, being represented solely by *Thermosipho melanesiensis* (Antoine et al. 1997), a member of the genus previously shown in shallow-water habitats (Huber et al. 1989), and *Desulfurobacterium thermoautotrophum* (L’Haridon et al. 1998), an obligately anaerobic hydrogen-utilizing sulfur-reducing bacterium. Many thermophilic microorganisms inhabiting deep-sea vents have been discovered since then.

Archaea generally comprise a larger fraction of microbial communities at vents compared with ocean sediments. Until recently organisms with lithotrophic sulfur respiration were not found in deep-sea thermal environments. The first thermophile of this physiological type found in a deep sea is *Ignicoccus pacificus* (Huber et al. 2000) which is an obligately anaerobic lithoautotroph utilizing only H<sub>2</sub>, elemental sulfur, and CO<sub>2</sub> as the energy substrate, the electron acceptor, and the carbon source, respectively. By using specific PCR primers, another *Ignicoccus* species has been found to host symbiotic representatives of a novel archaeal kingdom Nanoarchaeota in deep-sea vents (Huber et al. 2002; Hohn et al. 2002). Kashefi et al. (2002) obtained a Euryarchaeota *Geoglobus ahangari* from the Guaymas Basin hydrothermal system in 2002. A ferric iron-reducing thermophilic archaeon has been reported from an active “black smoker” in the Juan de Fuca Ridge. This was previously called strain 121, which is now tentatively named as “*Geogemma*

*barossii*" (Kashefi and Lovley 2003; Lovley et al. 2004). This obligate lithoautotroph, belonging to Desulfurococcales, utilizes molecular hydrogen alone and grows at 121°C. The heat-loving vent archaea *Pyrolobus fumarii* and strain 121 (*Geogemma barossii*) hold the global record for growth at high temperatures (113 and 121°C, respectively) (Blöchl et al. 1997; Kashefi and Lovley 2003). This temperature range is currently thought to represent the upper limit for life.

Among methanogens, a few members of Methanococcaceae have been recovered from hydrothermal vents of the Central Indian Ridge, representing new species of the genera *Methanothermococcus*, *Methanocaldococcus*, and *Methanotorris* (Takai et al. 2002, 2004; L'Haridon et al. 2003). *Methanococcus infernos* (Jeanthon et al. 1998) and *Methanococcus vulcanius* (Jeanthon et al. 1999) have been isolated from Mid-Atlantic Range and East Pacific Rise, respectively.

Various organotrophic hyperthermophilic archaea have been reported from deep-sea hydrothermal chimney, Suiyo Seamount, the Izu-Bonin Arc, Japan. *Aeropyrum camini* was the first obligately aerobic hyperthermophilic abyssal archaeon found in a deep-sea environment (Nakagawa et al. 2004). Representatives of Methanococcales, Methanopyrales, Archaeoglobales, and Thermococcales have been revealed from the analysis of hydrothermal and seafloor abyssal environment's 16S rRNA clone libraries which have also been detected by cultivation methods previously (Jeanthon 2000; Reysenbach et al. 2000a, b; Huber et al. 2002; Nercessian et al. 2003). An obligately anaerobic organotroph *Palaeococcus ferrophilus*, another new genus in the order Thermococcales (Takai et al. 2000), has also been isolated from a deep-sea chimney Myojin Knoll near Japan. Many novel representatives of the genus *Thermococcus* including *T. guaymasensis* (Canganella et al. 1997), *T. fumicolans* (Godfroy et al. 1996), *T. barophilus* (Marteinsson et al. 1999), *T. gammatolerans* (Jolivet et al. 2003), *T. coalescens* (Kuwabara et al. 2005), and *T. celericrescens* (Kuwabara et al. 2007) had been described from different hydrothermal vent sites. Representatives of some taxa like Thermoplasmatales (Reysenbach et al. 2000a, b; Huber et al. 2002) have been detected by molecular methods, which include obligate acidophiles that proliferate in inner parts of hydrothermal chimneys filled with acidic hydrothermal fluid at initial pH about 3.5. Among thermoacidophilic organisms, the representatives of Thermococcales have been detected from deep-sea vents by Prokofeva et al. (2005). A heterotrophic member of the ubiquitous, abundant, and apparently endemic deep-sea hydrothermal vent Euryarchaeota group DHVE2 had been cultivated. It shows optimum growth at a low pH and high temperature and the use of sulfur and iron as electron acceptors (Reysenbach et al. 2006).

The deep-sea hydrothermal vents are also habitat for various representatives of the domain bacteria. A species of the new genus *Persephonella*, which is related to *Aquifex* on the family level, has been isolated (Götz et al. 2002; Nakagawa et al. 2003). In 2003, Hoek et al. identified another member of Aquificaceae, *Hydrogenobacter thermolithotrophum*, by molecular techniques in a deep-sea hydrothermal environment. Two organisms growing in the temperature range from 55°C to 80°C and optimally at 70°C have been described from deep-sea thermal habitats (Jeanthon et al. 2002; Moussard et al. 2004). *Thermodesulfobacterium*



*hydrogenophilum* (Jeanthon et al. 2002) utilizes hydrogen and CO<sub>2</sub> as sources of energy and carbon, respectively. Another isolate *Thermodesulfatator indicus* (Moussard et al. 2004) exhibits very similar phenotypic characteristics but is only distantly related to Thermodesulfobacteriaceae (<90% 16S rRNA gene sequence similarity). *Deferribacter abyssi* (Miroshnichenko et al. 2003a) has been isolated from Suiyo Seamount hydrothermal chimney (Japan) and hot vents of the East Pacific Rise. The first thermophilic member of the family Geobacteraceae, *Geothermobacter ehrlichii*, has been isolated by Kashefi et al. (2003) from hydrothermal fluid of a Juan de Fuca Ridge deep-sea vent. The family included genera capable of dissimilatory ferric iron reduction (Lovley et al. 2004), and until recently this family consisted only of mesophilic species. This organism oxidizes volatile fatty acids, organic acids, and alcohols and reduces ferric iron to ferrous and nitrate to ammonium.

Among gram-negative thermophilic bacteria, Thermaceae constitutes one of the best-studied groups. Its first representative isolated from a deep-sea habitat under aerobic conditions is *Thermus thermophilus*, a strain of a widely occurring terrestrial species (Marteinsson 1999). Recently, several members of Thermaceae have been described from different deep-sea hydrothermal habitats, one of which was *Marinithermus hydrothermalis*. It is the only obligate aerobe among new isolates. The three other Thermaceae members *Oceanithermus profundus*, *Oceanithermus desulfurans* (Miroshnichenko et al. 2003b; Mori et al. 2004), and *Vulcanithermus mediatlanticus* (Miroshnichenko et al. 2003c) are the only microaerophiles in this family.

Representatives of the order Thermotogales have also isolated from deep-sea vent environments. A thermophilic, anaerobic, piezophilic, chemoorganotrophic sulfur-reducing bacterium, *Marinitoga piezophila*, had also been reported by Alain et al. (2002b). The growth of this bacterium is enhanced by hydrostatic pressure, the optimal pressure being 40 MPa (26 MPa pressure at sampling site). Another anaerobic and organotropic member of this order *Marinitoga hydrogenitolerans* that could tolerate 100% hydrogen had been described by Postec et al. (2005). A sulfur-reducing heterotrophic species of this genus, *M. okinawensis*, has been isolated by Nunoura et al. (2007b).

Epsilonproteobacteria represent an extremely abundant and diverse group in hydrothermal vents. This has been assessed by means of culture-independent molecular phylogenetic techniques. Members of this group dominate in microbial hydrothermal mats (Moyer et al. 1995; Longenecker and Reysenbach 2001) in the epibiotic microflora of deep-sea vent metazoans (Habbad et al. 1995; Campbell and Cary 2001) and diffuse flow hydrothermal fluids (Huber et al. 2003). Epsilonproteobacteria inhabiting deep-sea hot vents had been classified into six phylogenetic groups (A–F) by Corre and coworkers in 2001. The first representatives of Epsilonproteobacteria from deep-sea hydrothermal vents are *Nautilia lithotrophica* (Miroshnichenko et al. 2002) and *Caminibacter hydrogenophilus* (Alain et al. 2002a). *Caminibacter profundus* (Miroshnichenko 2004), which had been isolated from deep-sea hydrothermal system on the Mid-Atlantic Ridge, is an obligate lithoautotroph and microaerophile. Voordeckers et al. (2005) characterized another species *C. mediatlanticus*. Other



species belonging to the genus *Nautilia* (*N. nitratireducens*, *N. abyssi*, and *N. profundicola*) have also been recovered from hydrothermal vent ecosystems (Pérez-Rodríguez et al. 2010; Alain et al. 2009; Smith et al. 2008). Until the discovery of these representatives, Epsilonproteobacteria consisted of a single order Campylobacterales comprising two families: Campylobacteraceae and Helicobacteraceae (Vandamme and De Ley 1991). The representatives of the genera *Nautilia* and *Caminiibacter*, together with the uncultivated Epsilonproteobacteria, form a deep phylogenetic branch within the class Epsilonproteobacteria. Hence, Miroshnichenko and coworkers (2004) proposed a separate order Nautiliales of the subclass Epsilonproteobacteria with a single family Nautiliaceae. Today, this family includes three genera: *Nautilia*, *Caminiibacter*, and *Lebetimonas*, the latter one being represented by a single species *Lebetimonas acidiphila* (Takai et al. 2005). These Epsilonproteobacteria members are mostly chemolithotrophs and can utilize diverse electron acceptors. A novel thermophilic bacterium *Hydrogenimonas thermophilus* has been isolated from an in situ colonization system deployed in a superheated, deep-sea, hydrothermal vent emission at the Kairei Field on the Central Indian Ridge in the Indian Ocean. It has been classified within the previously uncultivated phylogroup {varepsilon}-Proteobacteria group A (Takai et al. 2003b). Two novel nitrifying bacteria belonging to {varepsilon}-Proteobacteria (*Nitratiruptor tergarcus* and *Nitratifactor salsuginis*) have been obtained from hydrothermal field Mid-Okinawa Trough, Japan (Nakagawa et al. 2005b). They are strict chemolithoautotrophs, which grow by respiratory nitrate reduction with H<sub>2</sub>, forming N<sub>2</sub> as a metabolic product.

Another phylogenetically remarkable organism isolated from a hydrothermal sample is *Caldithrix abyssi* (Miroshnichenko et al. 2003d). This anaerobic moderately thermophilic bacterium represents a new phylogenetic phylum. The similarity value of the 16S rRNA gene of *C. abyssi* with other known microorganisms does not exceed 82.3%.

Recently, novel sulfate reducers have been isolated from deep-sea hydrothermal chimneys. Two sulfate-reducing *Desulfothermus okinawensis* (Nunoura et al. 2007a) and *Desulfovibrio hydrothermalis* (Alazard et al. 2003) are heterotrophs, while *Desulfurobacterium atlanticum* (L'Haridon et al. 2006) is a chemolithoautotroph representing a new family Desulfurobacteraceae within the order Aquificales. A sulfur-oxidizing obligate chemolithoautotroph *Hydrogenivirga okinawensis* utilizes elemental sulfur or thiosulfate as an electron donor and nitrate or oxygen as an electron acceptor (Nunoura et al. 2008). Another strictly anaerobic sulfate reducer *Thermodesulfatator atlanticus* has recently been described from a deep-sea hydrothermal vent at the Rainbow site on the Mid-Atlantic Ridge (Alain et al. 2010).

Several other novel genera have been obtained from different hydrothermal vent ecosystems. A novel genus *Caloranaerobacter azorensis*, which is anaerobic and chemoorganotrophic, had been reported from a deep-sea hydrothermal chimney sample (Wery et al. 2001). A strict anaerobe, hydrogen-oxidizing chemolithoautotroph has been isolated from a black-smoker chimney in the Suiyo Seamount hydrothermal system and named as *Balnearium lithotrophicum* (Takai et al. 2003a). A lactic acid producing facultatively anaerobic, halotolerant, and moderately

thermophilic bacterium *Exiguobacterium profundum* had been isolated from hydrothermal vent of East Pacific Rise (Crapart et al. 2007). In 2008, Imachi et al. isolated an anaerobic and novel representative of the phylum Spirochaetes, *Exilispira thermophila*. *Sulfurivirga caldicuralii*, a microaerobic chemolithoautotroph capable of using thiosulfate or tetrathionate as a sole energy source, O<sub>2</sub> as the sole electron acceptor, and CO<sub>2</sub> as the sole carbon source, has been described from a shallow marine hydrothermal system (water depth 22 m) occurring in coral reefs off Taketomi Island, Okinawa (Takai et al. 2006a). Another bacterium *Thermosiphon affectus* representing a novel thermophilic anaerobic cellulolytic bacterium has been recovered from a hydrothermal vent system (Podosokorskaya et al. 2011). Some novel bacterial and archaeal thermophiles from deep-sea vents and their sites of isolation are presented in Tables 1.5 and 1.6.

The occurrence of fungi (filamentous fungi and yeasts) at deep-sea hydrothermal vents remains an underexplored topic. In contrast to surface environments, deep-sea environmental gene libraries have suggested that fungi are rare in high-pressure marine environments, and yeast forms dominate in these areas (Bass et al. 2007). This is because of the fact that fungal thermal tolerance is much lower than that of prokaryotes. Till now, no thermophilic yeasts are known from deep-sea vents (fungi are considered thermophilic if they grow at 50°C or higher temperatures and do not grow at 20°C or lower temperatures). Due to the presence of thermal gradient in hydrothermal fields (300–3°C), adequate conditions for mesophiles can also be found. Culture-dependent studies on the diversity of yeast and fungi in deep-sea environments discuss about those species that are mesophilic (Gadanhó and Sampaio 2005; Burgaud et al. 2009). Due to the development of culture-independent molecular techniques, the presence of fungi has also been directly reported in deep-sea environmental DNA samples. Sequence analysis of SSU ribosomal RNA genes reveals scarce fungal diversity, but some sequences are novel (Edgcomb et al. 2002; Lopez-Garcia et al. 2003, 2007). Bass et al. (2007) reported the presence of sequences affiliated to *Debaryomyces hansenii* and novel sequences closed to *Malassezia furfur* in hydrothermal sediments. Nagano et al. (2010) investigated the diversity of fungal communities in ten different deep-sea sediment samples by PCR-mediated internal transcribed spacer (ITS) regions of rRNA gene clone analysis and reported the predominance of deep-sea phylotypes belonging to Ascomycota in these environments.

### 1.2.1.3 Geothermally Heated Oil and Petroleum Reserves

An oil field is a region with an abundance of oil wells extracting crude oil (petroleum) from belowground. As oil reservoirs extend over a large area, multiple wells are scattered across this area for the extraction of oil. More than 40,000 oil fields are located throughout the Earth, on land and offshore. The largest are the Ghawar field in Saudi Arabia and the Burgan field in Kuwait. The initial reservoir pressure is often enough for the recovery of oil and gas from the well that flows freely to the surface (primary recovery). When this initial pressure is depleted, water is introduced into

**Table 1.5** List of novel thermophilic bacteria isolated from deep-sea hydrothermal vents

Genus/species	Site of isolation	T <sub>opt</sub> (°C)	References
<i>Thermosiphon melanensis</i>	Southwestern Pacific Ocean	70	Antoine et al. (1997)
<i>Desulfurobacterium hydrogenophilum</i>	Mid-Atlantic Ridge	70	L'Haridon et al. (1998)
<i>Marinotoga camini</i>	Mid-Atlantic Ridge	55	Wery et al. (2001)
<i>Caloranaerobacter azorensis</i>	Mid-Atlantic Ridge	65	Wery et al. (2001)
<i>Caldanaerobacter subterraneus</i>	Mid-Okinawa Trough	70	Sokolova et al. (2001)
<i>Hydrogenobacter acidophilus</i>	Vulcano Island	65	Stohr et al. (2001)
<i>Carboxydobrachium pacificum</i>	Okinawa Trough	70	Sokolova et al. (2001)
<i>Marinotoga piezophila</i>	East Pacific Rise	55	Alain et al. (2002b)
<i>Caminicella sporogenes</i>	East Pacific Rise	55–60	Alain et al. (2002c)
<i>Persephonella marina</i>	East Pacific Rise	73	Götz et al. (2002)
<i>Nautilia lithotrophica</i>	East Pacific Rise	53	Miroshnichenko et al. (2002)
<i>Caminibacter hydrogeniphilus</i>	East Pacific Rise	60	Alain et al. (2002a)
<i>Thermovibrio ruber</i>	Lihir Island, New Guinea	75	Huber et al. (2002)
<i>Tepidibacter thalassicus</i>	East Pacific Rise	50	Slobodkin et al. (2003)
<i>Caldithrix abyssi</i>	East Pacific Rise	60	Miroshnichenko et al. (2003d)
<i>Thermodesulfatator indicus</i>	Central Indian Ridge	70	Moussard et al. (2004)
<i>Deferribacter abyssi</i>	Mid-Atlantic Ridge	60–65	Miroshnichenko et al. (2003c)
<i>Clostridium caminithermale</i>	Atlantic Ocean Ridge	45	Brisbarre et al. (2003)
<i>Persephonella hydrogenophila</i>	Izu-Bonin Arc, Japan	70	Nakagawa et al. (2003)
<i>Desulfurobacterium crinifex</i>	Juan de Fuca Ridge	60–65	Alain et al. (2003)
<i>Balnearium lithotrophicum</i>	Izu-Bonin Arc, Japan	70–75	Takai et al. (2003a)
<i>Marinithermus hydrothermalis</i>	Izu-Bonin Arc, Japan	60–65	Sako et al. (2003)
<i>Oceanithermus profundus</i>	East Pacific Rise	60	Miroshnichenko et al. (2003a)
<i>Thermovibrio ammonificans</i>	East Pacific Rise	75	Vetriani et al. (2003)
<i>Tepidibacter formicigenes</i>	Mid-Atlantic Ridge	45	Urios et al. (2004)
<i>Caminibacter mediatlanticus</i>	Mid-Atlantic Ridge	55	Voordeckers et al. (2005)
<i>Hydrogenimonas thermophilus</i>	Central Indian Ridge	55	Takai et al. (2003b)
<i>Nitratiruptor tergarcius</i>	Mid-Okinawa Trough, Japan	55	Nakagawa et al. (2005b)
<i>Lebetimonas acidiphila</i>	Mariana Arc	50	Takai et al. (2005)
<i>Vulcanibacillus modesticaldus</i>	Mid-Atlantic Ridge	55	L'Haridon et al. (2006)
<i>Sulfurivirga caldicuralii</i>	Taketomi Island, Japan	50–55	Takai et al. (2006a)
<i>Thermaerobacter litoralis</i>	Kagoshima Prefecture, Japan	70	Tanaka et al. (2006)
<i>Desulfothermus okinawensis</i>	Southern Okinawa Trough	50	Nunoura et al. (2007a)
<i>Exiguobacterium profundum</i>	East Pacific Rise	45	Crapart et al. (2007)

(continued)

**Table 1.5** (continued)

Genus/species	Site of isolation	T <sub>opt</sub> (°C)	References
<i>Marinitoga okinawensis</i>	Southern Okinawa Trough	55–60	Nunoura et al. (2007b)
<i>Exilispira thermophila</i>	Okinawa Trough, Japan	50	Imachi et al. (2008)
<i>Clostridium tepidiprofundii</i>	East Pacific Rise	50	Slobodkina et al. (2008)
<i>Exilispira thermophila</i>	Okinawa Trough, Japan	50	Imachi et al. (2008)
<i>Hydrogenivirga okinawensis</i>	Southern Okinawa Trough	70–75	Nunoura et al. (2008)
<i>Thermosulfidibacter takaii</i>	Southern Okinawa Trough	70	Nunoura et al. (2008)
<i>Nautilia abyssi</i>	East Pacific Rise	60	Alain et al. (2009)
<i>Rhodothermus profundus</i>	East Pacific Rise	70	Marteinsson et al. (2010)
<i>Thermodesulfatator atlanticus</i>	Mid-Atlantic Ridge	65–70	Alain et al. (2010)
<i>Nautilia nitratireducens</i>	East Pacific Rise	55	Pérez-Rodríguez et al. (2010)
<i>Rhodothermus profundus</i>	East Pacific Rise	70	Marteinsson et al. (2010)
<i>Thermosiphon affectus</i>	Mid-Atlantic Ridge	70	Podosokorskaya et al. (2011)
<i>Thermosiphon globiformans</i>	Izu-Bonin Arc, Pacific Ocean	68	Kuwabara et al. (2011)

the injection well to pressurize the production well again. This is known as water flooding. It pushes the oil toward the production wells until oil production start again (secondary recovery).

These oil fields are one of the deep geological environments that support the existence of microorganisms. They occur at significant subsurface depth and are characterized by a high in situ temperature. Thus, these geothermally heated petroleum reservoirs represent unique biotopes that constitute ecological niches providing suitable conditions to support thermophiles (Stetter et al. 1993). They have attracted the great interest of researchers for the isolation of novel thermophilic microorganisms (Greene et al. 1997; Bonch-Osmolovskaya et al. 2003; Magot et al. 2000; Salinas et al. 2004a, b; DiPippo et al. 2009; Jayasinghearachchi and Lal 2011).

#### Diversity of Thermophilic Microorganisms in Oil Fields and Petroleum Reservoirs

Physiological types of microorganisms that have been isolated from these biotopes include heterotrophs (Takahata et al. 2001; DiPippo et al. 2009), sulfate reducers (Tardy-Jacquenod et al. 1996; Stetter et al. 1993; Miranda-Tello et al. 2003), sulfidogens (L'Haridon et al. 1995; Stetter et al. 1993), fermentative bacteria (Davey et al. 1993; Grassia et al. 1996; Miranda-Tello et al. 2007), manganese and iron

**Table 1.6** List of novel thermophilic archaea isolated from deep-sea hydrothermal vents

Genus/species	Site of isolation	T <sub>opt</sub> (°C)	References
<i>Methanocaldococcus jannaschii</i>	Guaymas Basin	86	Zhao et al. (1988), Jones et al. (1983)
<i>Archaeoglobus profundus</i>	Mid-Atlantic Ridge	92	Burggraf et al. (1990)
<i>Methanopyrus kandleri</i>	Guaymas Basin	110	Kurr et al. (1991)
<i>Pyrococcus abyssi</i>	North Fiji Basin	96	Erauso et al. (1993)
<i>Thermococcus profundus</i>	Mid-Okinawa Trough	90	Kobayashi et al. (1994)
<i>Thermococcus chitonophagus</i>	East Pacific Rise	93	Huber et al. (1995),
<i>Thermococcus hydrothermalis</i>		85	Godfroy et al. (1997)
<i>Thermococcus funicolans</i>	North Fiji Basin	85	Godfroy et al. (1996)
<i>Archaeoglobus veneficus</i>	Mid-Atlantic Ridge	–	Huber et al. (1997)
<i>Thermococcus guaymasensis</i>	Guaymas Basin	80–90	Canganella et al. (1997)
<i>Methanocaldococcus infernus</i>	Mid-Atlantic Ridge	85	Jeanthon et al. (1998)
<i>Thermococcus siculi</i>	Mid-Okinawa Trough	90	Grote et al. (1999)
<i>Methanocaldococcus vulcanius</i>	East Pacific Rise	80	Jeanthon et al. (1999)
<i>Methanocaldococcus fervens</i>	Guaymas Basin	–	Jeanthon et al. (1999)
<i>Thermococcus barophilus</i>	Mid-Atlantic Ridge	95	Marteinsson et al. (1999)
<i>Pyrococcus glycovorans</i>	East Pacific Rise	95	Barbier et al. (1999)
<i>Palaeococcus ferrophilus</i>	Ogasawara-Bonin Arc., Japan	83	Takai et al. (2000)
<i>Ignicoccus pacificus</i>	East Pacific Rise	90	Huber et al. (2000)
<i>Methanothermococcus okinawensis</i>	Mid-Okinawa Trough	60–65	Takai et al. (2000)
<i>Geoglobus ahangari</i>	Guaymas Basin	88	Kashefi et al. (2002)
<i>Thermococcus gammatolerans</i>	Guaymas Basin	88	Jolivet et al. (2003)
<i>Geogemma barossii</i>	Juan de Fuca Ridge	105–107	Kashefi and Lovley (2003)
<i>Methanocaldococcus indicus</i>	Central Indian Ridge	85	L'Haridon et al. (2003)
<i>Methanoterris formicicus</i>	Central Indian Ridge	85	Takai et al. (2004)
<i>Aeropyrum camini</i>	Izu-Bonin Arc, Japan	85	Nakagawa et al. (2004)
<i>Thermococcus coalescens</i>	Suiyo Seamount	87	Kuwabara et al. (2005)
<i>Thermococcus celericrescens</i>	Suiyo Seamount, Western Pacific Ocean	80	Kuwabara et al. (2007)
<i>Thermococcus thio reducens</i>	Mid-Atlantic Ridge	83–85	Pikuta et al. (2007)
<i>Pyrococcus yayanosii</i>	Mid-Atlantic Ridge	98	Birrien et al. (2011)

reducers (Greene et al. 1997), methanogens (Rožanova et al. 1997; Ng et al. 1989; Cheng et al. 2007), acetogens (Davydova-Charakhchyan et al. 1993), and many more. Most of them are anaerobes, including a few aerobes (Nazina et al. 2001, 2005). Table 1.7 lists the novel thermophilic microorganisms isolated from various geothermal oil and petroleum reservoirs. Apart from culture-based approaches, different molecular methods, like reverse genome probing, hybridization with functional

**Table 1.7** List of novel thermophiles isolated from geothermally heated oil and petroleum reserves

Genus/species	Location	T <sub>opt</sub> (°C)	Comments	References
<i>Thermoanaerobacter brockii</i> subsp. <i>lactiethylicus</i>	Deep subsurface French oil well	55–60	Strict anaerobe	Cayol et al. (1995)
<i>Thermotoga elfii</i>	African oil-producing well	66	Strict anaerobe	Ravot et al. (1995)
<i>Desulfotomaculum thermocisternum</i>	North Sea oil reservoir, UK	62	Sulfate reducer	Nilsen et al. (1996)
<i>Anaerobaculum thermoterrenum</i>	–	55	Anaerobic citrate fermentor	Rees et al. (1995)
<i>Deferribacter thermophilus</i>	Beatrice oil field in the North Sea, UK	60	Anaerobe	Greene et al. (1997)
<i>Thermosipho geolei</i>	Oil reservoir in Siberia (Russia)	70	Anaerobic S <sup>0</sup> reducer	L'Hardon et al. (2001)
<i>Thermotoga petrophila</i>	Kubiki oil reservoir in Niigata, Japan	Range 47–88	Strictly anaerobic heterotroph	Takahata et al. (2001)
<i>Thermotoga naphthophila</i>	Kubiki oil reservoir in Niigata, Japan	Range 48–86	Strictly anaerobic heterotroph	Takahata et al. (2001)
<i>Geobacillus subterraneus</i>	Oil fields in Russia, Kazakhstan, and China	–	Moderately thermophile, aerobe	Nazina et al. (2001)
<i>Geobacillus uzonensis</i>	Oil fields in Russia, Kazakhstan, and China	–	Moderately thermophile, aerobe	Nazina et al. (2001)
<i>Petrotoga olearia</i>	Continental oil reservoir in Western Siberia	55	Anaerobe	L'Hardon et al. (2002)
<i>Petrotoga sibirica</i>	Continental oil reservoir in Western Siberia	55	Anaerobe	L'Hardon et al. (2002)
<i>Desulfovibrio capillatus</i>	Mexican oil field	40	Sulfate reducer	Miranda-Tello et al. (2003)
<i>Garcicella nitratireducens</i>	Gulf of Mexico	55	Anaerobic nitrate and thiosulfate reducer	Miranda-Tello et al. (2003)

<i>Mahella australiensis</i>	Australian terrestrial oil reservoir	50	Anaerobe	Salinas et al. (2004b)
<i>Petrobacter succinatimandens</i>	Australian terrestrial oil reservoir	55	Aerobic nitrate reducer	Salinas et al. (2004a)
<i>Petrotoga mexicana</i>	Gulf of Mexico	55	Anaerobic S <sup>0</sup> reducer	Miranda-Tello et al. (2004)
<i>Caldanaerobacter subterraneus</i>	Oil fields of France	–	Anaerobe	Fardeau et al. (2004)
<i>Thermovirga lienii</i>	North Sea oil reservoir, UK	58	Anaerobe	Dahle and Birkeland (2006)
<i>Methermicrococcus shengliensis</i>	Shengli oil field, China	65	Methylotroph	Cheng et al. 2007
<i>Petrotoga halophila</i>	Oil-producing well in Congo, West Africa	60	Fermentative	Miranda-Tello et al. (2007)
<i>Kosmotoga olearia</i>	Troll B oil platform, North Sea	65	Heterotroph	DiPippo et al. (2009)
<i>Oceanotoga teriensis</i>	Oil production well, Bombay High, India	55–58	Chemoorganotroph	Jayasinghearachchi and Lal (2011)



gene probes, 16S rDNA analysis, and immunological techniques, have been used to assess physiological and genetic diversity of thermophiles in the oil fields (Christensen et al. 1992; Leu et al. 1998; Voordouw et al. 1996, 1992).

*Pyrococcus* and *Thermococcus* spp. have been found in oil reservoirs (Stetter et al. 1993). Both of them derive energy by fermentation of peptides, amino acids, and sugars, forming fatty acids, CO<sub>2</sub>, and H<sub>2</sub> (Fiala and Stetter 1986). *Pyrococcus furiosus* is able to ferment pyruvate, forming acetate, H<sub>2</sub>, and CO<sub>2</sub> (Schäfer and Schönheit 1992). Some species of archaeal coccoid sulfate reducers occur within hot oil reservoirs and may be responsible for H<sub>2</sub>S production or “reservoir souring” there (Stetter et al. 1993). *Archaeoglobus fulgidus* and *Archaeoglobus lithotrophicus* fulfill their energy requirements by reduction of SO<sub>4</sub><sup>2-</sup> by H<sub>2</sub>. *Archaeoglobus profundus* is an obligate heterotroph (Beeder et al. 1994).

The novel genera and species *Thermodesulforhabdus norvegicus* (Beeder et al. 1995) and *Desulfacinum infernum* (Rees et al. 1995) have been isolated from deep, hot oil fields. Oil fields and geothermal springs have commonly yielded moderately thermophilic members of the genus *Desulfotomaculum*, including *Desulfotomaculum geothermicum* (Daumas et al. 1988), *Desulfotomaculum australicum* (Love et al. 1993), and *Desulfotomaculum kuznetsovii* (Nazina et al. 1987). These sulfate reducers can utilize a wide substrate spectrum of low-molecular-weight alcohols and organic acids.

Species of the genera *Geotoga* and *Petrotoga* have been retrieved only from deep oil reservoirs. *Petrotoga miotherma* (Davey et al. 1993) and *Petrotoga mobilis* (Lien et al. 1998) have been isolated from petroleum reservoirs from Oklahoma and Texas and from the North Sea, respectively. Strictly anaerobic *Petrotoga olearia* and *Petrotoga sibirica* have been described from continental oil reservoir in Western Siberia by the 16S rDNA sequence analysis (L’Haridon et al. 2002). *Petrotoga mexicana* and *Petrotoga halophila* have been found in oil reservoirs of Gulf of Mexico (Miranda-Tello et al. 2004) and Congo, West Africa (Miranda-Tello et al. 2007), respectively. Recent microbiological studies performed on oil–water mixtures from different high-temperature strata of the Samotlor oil fields (Western Siberia) have identified phylogenetically diverse thermophilic organisms (Slobodkin et al. 1999; Jeanthon et al. 2000). Among them, novel species of the genera *Thermococcus* and *Thermosipho* have been isolated and characterized (L’Haridon et al. 2001; Miroshnichenko et al. 2001).

Among the aerobic, thermophilic, spore-forming bacteria, members of the genus *Geobacillus* (*G. subterraneus* and *G. uzenensis*) have been isolated (Nazina et al. 2000). *Geobacillus jurassicus* and *G. stearothermophilus* have also been described from oil reservoirs of China. These are aerobic, gram-positive, rod-shaped, moderately thermophilic chemoorganotrophs capable of growing on various sugars, carboxylic acids, and crude oil (Nazina et al. 2005).

Both molecular and culture-based methods have been used to characterize prokaryotic microorganisms associated with high-temperature, sulfur-rich oil reservoirs in California. Heterotrophic enrichments from all sites yielded sheathed rods (*Thermotogales*), pleomorphic rods resembling *Thermoanaerobacter*, and *Thermococcus*-like isolates. The major autotrophs recovered from inorganic enrichments include

isolates closely related to *Methanobacterium*, *Methanococcus*, and *Methanoculleus* species. Sequence analysis of 16S rDNA libraries generated from total community DNA yield *Thermoanaerobacter*, *Thermococcus*, *Desulfovibrio*, *Aminobacterium*, *Acidaminococcus*, *Pseudomonas*, *Halomonas*, *Acinetobacter*, *Sphingomonas*, *Methylobacterium*, and *Desulfomicrobium*, suggesting the widespread distribution of sulfur-utilizing and methane-producing thermophilic microorganisms in these oil reservoirs (Orphan et al. 2000).

Microbial biodiversity in the formation waters of the Samotlor high-temperature oil reservoir (Western Siberia, Russia) has been demonstrated by the radioisotopic, cultural, and molecular methods. Enrichment cultures indicate the presence of diverse physiological groups representing aerobic and anaerobic thermophiles and hyperthermophiles, primarily the representatives of *Thermotoga*, *Thermoanaerobacter*, *Geobacillus*, *Petrotoga*, *Thermosipho*, and *Thermococcus*. Oligonucleotide microchip analyses also revealed the presence of several groups of microorganisms that escaped cultivation, which included the representatives of *Aquificales* and members of *Desulfurococcus* and *Thermus*, which have been unknown in this habitat till now (Bonch-Osmolovskaya et al. 2003).

Thermotolerant sulfidogenic communities have been examined in the production waters of onshore oil fields in North-Eastern India. Most of the sulfur-reducing bacteria have been affiliated to the members of *Desulfovibrio*, *Desulfomicrobium*, *Desulfotomaculum*, and *Desulfobulbus* based on 16S rDNA sequence analysis. Most of the thiosulfate-reducing isolates have been affiliated with the phylum Firmicutes, including *Clostridium* and *Fusibacter*, and also with the phylum Proteobacteria, including the genera *Enterobacter* and *Citrobacter* (Agrawal et al. 2010). Anaerobic sulfate and thiosulfate reducers *Desulfovibrio capillatus* and *Garciella nitratreducens* have also been described from Mexican oil fields (Miranda-Tello et al. 2003).

#### 1.2.1.4 Solar-Heated Soils/Sediments

Thermophilic representatives of bacteria and fungi have been isolated from solar-heated soils and sediments. The main source-producing heat in these habitats is solar heat radiation (Geiger 1965). There are over 200 identified bacterial genera, and a single soil sample may have over 2,000 genetically distinct bacteria (Torsvik et al. 1990). There has been widespread occurrence of thermophilic bacteria, filamentous fungi, and yeast in solar-heated soils at the temperatures reaching 70°C. Thermophilic spore formers belonging to *Bacillus*, *Clostridium*, and *Thermoactinomyces* have been isolated from soils. These include aerobes and facultative anaerobes (*Bacillus stearothermophilus*, *B. acidocaldarius*, *B. caldotenax*, *Thermoactinomyces vulgaris*) and anaerobes (*Clostridium thermocellum* and *C. thermohydrosulfuricum*) (Zeikus 1979). Touzel et al. (2000) isolated a novel aerobic, thermophilic, xylanolytic, spore-forming bacterium from a farm soil in France. This gram-negative bacillus *Thermobacillus xylanilyticus* shares 91.15% sequence similarity with *Bacillus viscosus* and grows at temperatures up to 63°C.

Another novel thermoalkaliphilic obligately anaerobe has been isolated from a humid soil sample in Kenya. This gram-positive bacterium *Anaerobranca gottschalkii* grows optimally at pH 9.5 and 50–55°C (Prowe and Antranikian 2001). Novel thermophilic actinobacteria *Amycolatopsis eurytherma*, *Saccharopolyspora flava*, and *Saccharopolyspora thermophila* have been isolated from soil (Kim et al. 2002; Lu et al. 2001). Yabe et al. (2011) isolated two novel thermophilic spore-forming bacteria belonging to novel family Thermogemmatissporaceae within the class Ktedonobacteria, from fallen leaf samples in the soil. These two bacteria *Thermogemmatisspora onikobensis* and *T. foliorum* grow at the temperature optima of 60–65°C and show maximum 16S rDNA homology to *Thermosporothrix hazakensis*. Zucchi et al. (2012) isolated two thermophilic actinomycetes *Amycolatopsis thermophila* sp. and *Amycolatopsis viridis* sp., thermophilic from arid soil. *Chthonomonas calidirosea* gen. nov., sp. nov., is an aerobic, pigmented, thermophilic bacterium belonging to a novel bacterial class, *Chthonomonadetes* classis nov. It was isolated from geothermally heated soil at Hell's Gate, Tikitere, New Zealand. On the basis of 16S rRNA gene analysis, this represents the first representative of this new class in the newly described phylum *Armatimonadetes*, which was formerly called as candidate division OP10 (Lee et al. 2011).

By direct solar heating, the soil surface temperature frequently rises in summer to levels allowing the development of thermophilic fungi. Here, fungi exist either as resting propagules or as active mycelia depending on the availability of nutrients and favorable environmental conditions. Widespread occurrence of thermophilic fungi in soils has been established by many studies (Taber and Pettit 1975; Awao and Mitsugi 1973; Ward and Cowley 1972; Minoura et al. 1973). Mouchacca (1995) reviewed the isolation of many thermophilic fungi [*Scytalidium thermophilum*, *Thermomyces lanuginosus* (*Humicola lanuginosa*), *Myceliophthora thermophila*, *Malbranchea cinnamomea*, *Chaetomium thermophile*, *Melanocarpus albomyces*, *Talaromyces thermophilus*, *Thermoascus aurantiacus*, *Rhizomucor pusillus*, *Myceliophthora fergusii* (*Corynascus thermophilus*), *Myriococcum thermophilum*, *Thielavia terrestris* (*Acremonium alabamense*), *Melanocarpus albomyces*, *Rhizomucor miehei*, *Rhizomucor pusillus*, *Thielavia terrestris*, *Talaromyces byssochlamydoides*, *Talaromyces emersonii*, *Talaromyces thermophilus*] from solar-heated and desert soils. Few thermophilic and thermotolerant organisms like *Chaetomium senegalense* (ascomycetes) and *Myceliophthora fergusii* (anamorphic ascomycetes) have reported for the first time from India (Salar and Aneja 2006). DNA sequence and fingerprinting approaches have also been used successfully to profile and characterize fungal communities in soils (O'Brien et al. 2005; Pérez-Piqueres et al. 2006; Anderson et al. 2008; Bates and Garcia-Pichel 2009).

Soils are the appropriate habitats for the isolation of thermophilic yeasts. *Candida thermophila* has been isolated from soil samples in Korea (Shin et al. 2001). Peter et al. (2007) observed ascospore formation in this yeast strain for the first time and therefore named its teleomorph as *Ogataea thermophila*. Two novel thermotolerant methylotrophic yeast species *Ogataea chonburiensis* and *Ogataea nakhonphanomensis* have been isolated from a tree exudate and soil collected in Thailand (Limtong et al. 2008). Three strains of a thermotolerant methylotroph

*Pichia thermomethanolica* have also been isolated from soils of Thailand (Limtong et al. 2005), which are later renamed as *Ogataea thermomethanolica* (Limtong et al. 2008).

## 1.2.2 Man-Made Thermophilic Habitats

Man-made thermophilic habitats include acid mine drainage and acidic effluents, self-heated compost piles, biological wastes, and waste treatment plants. These are comparatively lower temperature habitats, as compared to the natural habitats, and are ideal for the isolation of moderate and extreme thermophiles.

### 1.2.2.1 Acid Mine Drainage and Acidic Effluents

Acid mine drainage (AMD) or acid rock drainage (ARD) refers to outflow of highly acidic (pH 2.3–6.5), metal-rich fluids from metal mines or coal mines. These fluids are hot (temperature reaching up to as high as 70°C) because they are generated due to exothermic metal sulfide oxidation reactions. The predominant metal sulfide mineral in most rocks is pyrite (FeS<sub>2</sub>) which is found in association with many commercially valued metals like Au, Ag, Cu, Zn, and Pb. These metals are present as impurities in pyrite or as sulfide minerals associated with pyrite, such as chalcopyrite (CuFeS<sub>2</sub>), sphalerite (ZnS), and galena (PbS). Variable amounts of pyrites are found in the coal deposits also. Mining increases the surface area of these ores exposed to air and water. After being exposed to water and air, oxidation of metal sulfides within the surrounding rock occurs at a faster rate, leading to the formation of water enriched in sulfate, aluminum, and heavy metals (Johnson and Hallberg 2003; Ackil and Koldas 2006). This reaction serves as template for the several oxidation reactions which contributes to further increase in acidity.

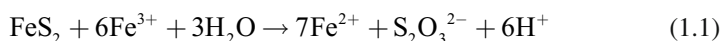
Despite the extreme acidity, heat, and high concentrations of sulfate and toxic metals, a diverse range of microorganisms populate this environment. These organisms are chemoautotrophs and utilize sulfide (from ores); CO<sub>2</sub>, O<sub>2</sub>, and N<sub>2</sub> (from air); and phosphate (liberated by water–rock interaction) as electron donors. Microbial activity increases the rate of AMD formation and may be responsible for the bulk of AMD generated. DNA-based studies on the diversity of microorganisms in these environments have provided insights into many acidophilic, metal-tolerant, and thermophilic prokaryotes. Eukaryotes (protists, fungi, and yeasts) are abundant and important in some parts of acid systems like solfataras, but diversity of eukaryotes in AMD and acidic effluents still remains an underexplored topic. Some of the acid mine drainage sites include Storwartz mine, Norway; Ynysarwed, Wales; Bull House, England; Wheal Jane, England; Killingdal mine dump, Norway; King's mine stream, Norway; Parys mine, Wales; Argo Tunnel, Idaho Springs, Colorado, USA; Iron Mountain Mine, USA; Monday Creek, Ohio; Davis Pyrite Mine, Massachusetts; Hughes bore hole, Pennsylvania, Aznalcollar mine, Spain; and Rio Tinto River, Spain.

## Chemistry of Acid Mine AMD

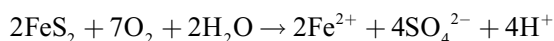
The formation of AMD from pyrite can be represented in three basic chemical reactions: pyrite oxidation, ferrous oxidation, and iron hydrolysis (Johnson 2003).

### Reaction 1: Pyrite Oxidation

Pyrite oxidizes spontaneously, with either molecular oxygen or ferric iron acting as the oxidant

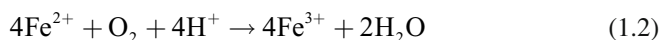


Or



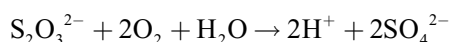
This reaction is abiotic and may occur in anaerobic as well as aerobic environments depending on the oxidant.

### Reaction 2: Ferrous Oxidation



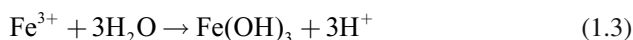
This reaction requires oxygen and is primarily biological in acidic ( $\text{pH} < 4$ ) waters. The rate of this reaction is slow at low pH; thus, it may limit the rate of AMD generation. However, microorganisms accelerate this reaction rate, thus can determine the rate of pyrite dissolution.

Some bacteria and archaea can also oxidize thiosulfate, the initial sulfur by-product of pyrite oxidation (reaction 1), and other reduced inorganic sulfur compounds (RISCs) and elemental sulfur, producing sulfuric acid as follows:



### Reaction 3: Iron Hydrolysis

Further acidity results from the hydrolysis of ferric iron:



## Diversity of Microorganisms in AMD and Acidic Effluents

In acid mine drainage and effluents from different sites, variations occur in the temperature, ionic strength, and pH, yielding different communities characterized by different microorganisms, but all sites are restricted to a few species. This can be due to the limited number of energy-deriving reactions available in AMD environments.

Photosynthesis is an important source of energy and fixed carbon regions of AMD systems exposed to sunlight (Hiraishi et al. 2000). However, in subsurface ARD and AMD systems, the 4 major metabolic groups are detected. They are lithoautotrophs that oxidize  $\text{Fe}^{2+}$  and  $\text{S}^-$ , organoheterotrophs that utilize carbon produced by

lithoautotrophs, lithoheterotrophic that are iron and S<sup>0</sup> oxidizers, and anaerobes that couple oxidation of sulfur or organic carbon to Fe<sup>3+</sup> reduction.

Among bacteria, major representatives present in the AMD community lie within Proteobacteria, Nitrospira, Acidobacteria, and firmicutes. *Acidithiobacillus* spp. (formerly *T. ferrooxidans*, *Thiobacillus caldus*) (Kelly and Wood 2000) and *Thiobacillus* spp. are primarily responsible for acid mine drainage. They belong to  $\gamma$ -Proteobacteria. The moderately thermophilic *Acidithiobacillus caldus* (formerly *Thiobacillus caldus*) is a sulfur oxidizer. “*Leptospirillum ferrooxidans*” group within firmicutes division is commonly detected in AMD systems. *Leptospirillum* isolates and environmentally derived clones cluster within one of three phylogenetically distinct groups (I, II, and III) (Bond et al. 2000). Two species have been named: *L. ferrooxidans* (group I) and *L. ferriphilum* (group II) (Hippe 2000; Coram and Rawlings 2002). Group III *Leptospirillum* has only been detected via clone library analysis only (Bond et al. 2000). *Leptospirillum* isolates have been obtained from AMD environments characterized by a wide range of temperatures and pH. *L. ferrooxidans* DMZ2705 (group I) has been reported to grow in the pH range of 1.3–4.0 and an upper temperature limit of 55°C (Hippe 2000; Johnson and Roberto 1997).

Another large bacterial group named *Sulfobacillus*, which is represented by *S. thermosulfidooxidans* and *S. acidophilus*, is separate from the *Alicyclobacillus* lineage. *Sulfobacillus* spp. are moderate thermophiles and have a broad range of physical growth regimes. Some isolates that are capable of growth up to 65°C have also been isolated (Atkinson et al. 2000). They either oxidize ferrous iron or reduce ferric iron, depending on prevailing concentrations of dissolved oxygen (Bridge and Johnson 1998).

Archaeal lineages reported from AMD environments are restricted to the Thermoplasmatales and the Sulfolobales. Clones within the Thermoplasmatales (e.g., *Thermoplasma acidophilum* and *Thermoplasma volcanium*) have been detected in clone libraries created from samples collected. *T. acidophilum* and *T. volcanium* have moderately thermophilic (45–67°C) temperature and pH ranges (Darland et al. 1970; Segerer et al. 1988) typical of AMD.

One member of the Sulfolobales, *Metallosphaera prunae*, has been detected in AMD environments. Another species of this genus *Metallosphaera sedula* is a powerful oxidizer of pyrite, chalcopyrite, and sphalerite, forming sulfuric acid and solubilizing heavy metals. Two other Sulfolobales genera, *Acidianus* and *Sulfolobus*, have been isolated from AMD and also from other geothermal acidic environments. *Sulfolobus* spp. are strict aerobes growing autotrophically by oxidation of S<sup>0</sup>, S<sup>2-</sup>, and H<sub>2</sub>, forming sulfuric acid or water as end product. *Sulfolobus brierleyi* (now renamed as *Acidianus brierleyi*) and *Sulfolobus metallicus* are able to grow by leaching sulfide ores (Brierley and Brierley 1973; Huber and Stetter 1991). Several *Sulfolobus* isolates are facultative or obligate heterotrophs (Brock 1978).

They are able to reduce ferric and molybdate under microaerophilic conditions, and their growth requires low ionic strength (Brierley and Brierley 1973). *Acidianus*, like *Sulfolobus*, is able to grow by oxidation of S<sup>0</sup>, sulfides, H<sub>2</sub>, and organic matter but is able to grow anaerobically by reduction of elemental sulfur

**Table 1.8** List of thermoacidophiles isolated from acid mine drainage and acidic effluents

Thermal category	Organism	Phylogenetic affiliation	Comments
Moderate thermophile	<i>Sulfobacillus acidophilus</i>	Firmicutes	Iron oxidizer
	<i>S. thermosulfidooxidans</i>	Firmicutes	Iron oxidizer
	<i>Acidimicrobium ferrooxidans</i>	Actinobacteria	Iron oxidizer/ reducer
	<i>Leptospirillum thermoferrooxidans</i>	Nitrospira	Iron oxidizer
	<i>Acidithiobacillus caldus</i>	β/γ-Proteobacteria	Sulfur oxidizer
	<i>Hydrogenobacter acidophilus</i>	Aquificales	Sulfur oxidizer
	<i>Alicyclobacillus</i> spp.	Firmicutes	Heterotrophic
	<i>Thermoplasma acidophilum</i>	Thermoplasmatales	Heterotrophic
	<i>Th. volcanicum</i>	Thermoplasmatales	Heterotrophic
	Extreme thermophile	<i>Acidianus brierleyi</i>	Sulfolobales
<i>A. infernus</i>		Sulfolobales	Iron oxidizer
<i>A. ambivalens</i>		Sulfolobales	Iron oxidizer
<i>Metallosphaera sedula</i>		Sulfolobales	Iron oxidizer
<i>Sulfolobus shibatae</i>		Sulfolobales	Sulfur oxidizer
<i>Sf. solfataricus</i>		Sulfolobales	Sulfur oxidizer
<i>Sf. hakonensis</i>		Sulfolobales	Sulfur oxidizer
<i>Sf. metallicus</i>		Sulfolobales	Sulfur oxidizer
<i>Metallosphaera prunae</i>		Sulfolobales	Sulfur oxidizer
<i>Sulfurococcus mirabilis</i>		Sulfolobales	Sulfur oxidizer
<i>Sulfolobus acidocaldarius</i>		Sulfolobales	Sulfur oxidizer
<i>Stygiolobus azoricus</i>		Sulfolobales	Obligate anaerobe
<i>Acidilobus aceticus</i>		Sulfolobales	Obligate anaerobe

From Johnson and Hallberg (2003)

with H<sub>2</sub> as electron donor (Seeger et al. 1985). Environmental clones closely related to *Ferromicrobium acidophilus* have been recovered from the Richmond Mine AMD system (43°C) (Bond et al. 2000). *Acidimicrobium ferrooxidans* has been cultivated at temperatures between 34 and 57°C (Clark and Norris 1996) and has been reported from a surprisingly diverse range of environments including AMD. Table 1.8 enlists some thermoacidophiles isolated from AMD and acidic effluents.



### 1.2.2.2 Biological Wastes and Waste Treatment Plants

Waste materials are the unwanted or unusable materials which are disposed or are intended to be disposed. Litter is waste which has been disposed of improperly, particularly waste which has been carelessly disposed of in plain sight. Waste Management Licensing Regulations 1994 define waste as any substance or object which the producer or the person in possession of it discards or intends or is required to discard. As defined by Pongrácz and Pohjola (2004), waste is a man-made thing that has no purpose or is not able to perform with respect to its purpose. There are many waste types defined by modern systems of waste management including municipal solid waste (MSW), construction waste and demolition waste (C&D), institutional waste, commercial waste and industrial waste (IC&I), medical waste, hazardous waste, radioactive waste, and electronic waste and biodegradable waste. Waste may also be divided into solid waste or liquid waste. Solid waste includes household waste or municipal waste (food and kitchen waste, paper waste, and hazardous waste), industrial waste (factory waste, waste from mills and mine, paper and pulp effluents), agricultural waste, sewage sludge, and biomedical waste. Liquid waste includes wastewater discharged by domestic residences, commercial properties, industry, and/or agriculture and can encompass a wide range of potential contaminants and concentrations. Sewage includes domestic, municipal, or industrial liquid waste products disposed of, usually via a pipe or sewer.

Vast amounts of wastes require safe disposal. As defined by Pongrácz and Pohjola (2004), waste management is control of waste-related activities with the aim of protecting the environment and human health and resources conservation. Waste-related activities include waste-creating processes, waste handling, as well as waste utilization. The main aim of waste management besides waste evasion is turning wastes to non-wastes Pongrácz and Pohjola (2004). There are different kinds of waste management methods depending upon the kind of waste (organic or inorganic). The simplest way to handle solid waste disposal at the lowest direct cost is in landfills. In this procedure, solid wastes are deposited in low-lying and low-value land. Anaerobic and facultative microorganisms attack the organic compounds in the waste and degrade them (Senior 1995). Another method of disposal of solid domestic and agricultural waste is composting. This topic shall be dealt separately later in this chapter. Other methods include incineration, plasma gasification, anaerobic digestion, and thermophilic aerobic digestion (TAD). Liquid waste treatment methods depend on type, composition, and origin of liquid waste. Sewage waste can be treated close to where it is created (in septic tanks, biofilters, or aerobic treatment systems) or collected and transported via a network of pipes and pump stations to a municipal treatment plant. Conventional sewage treatment may involve three stages, called primary (pretreatment), secondary, and tertiary treatment. Secondary treatment is the major step of sewage treatment which relies upon use of indigenous microorganisms. It removes dissolved and suspended biological matter. Secondary treatment systems are classified as fixed-film or suspended-growth systems. Fixed-film or attached growth systems include filter beds (trickling filters) and rotating biological contactors, where the microbial biomass grows on media and the sewage passes over its surface. Suspended-growth systems include activated sludge, which involves

introduction of air or oxygen to the mixture of sludge (residual, semisolid material left from primary treated sewage), sewage, and microorganisms in an aeration tank to develop biological floc which reduces organic content of the sewage. Another secondary treatment method is anaerobic digester, which is a slow anaerobic process that decreases organic content of the sewage.

### Diversity of Thermophilic Microorganisms in Biological Wastes and Waste Treatment Plants

A vast array of thermophiles has been isolated from different anthropogenic wastes and treatment plants. Microorganisms have been described from various water sources, such as wastewaters from industrial plants, factory effluents, and wastewater treatment plants (Carreto et al. 1996, Menes et al. 2002, Kaksonen et al. 2007; Tan and Guodong 2010). A slightly halotolerant gram-positive thermophilic bacterium, *Rubrobacter xylanophilus*, has been isolated from a thermally polluted industrial runoff near Salisbury, United Kingdom. Its optimum growth temperature is approximately 60°C (Carreto et al. 1996). Cann et al. 2001 isolated two anaerobic polysaccharide-degrading thermophiles from the leachate of a waste pile from a canning factory in Hoopston, East Central Illinois, USA. *Thermoanaerobacterium polysaccharolyticum* reduces thiosulfate to sulfide, whereas *Thermoanaerobacterium zae* is unable to reduce thiosulfate. The temperature optimum of growth for both of these anaerobes is 65–68°C. *Anaerobaculum mobilis*, a novel anaerobic, moderately thermophilic, peptide-fermenting sulfur reducer with the optimum temperature range 55–60°C, has been isolated from an anaerobic wool-scouring wastewater treatment lagoon (Menes et al. 2002). Novel thermophilic sulfur reducers *Desulfurispora thermophila* and *Desulfotomaculum alcoholivorax* have been isolated from a sulfidogenic fluidized-bed reactor treating acidic metal- and sulfate-containing water. These fermentative bacteria grow optimally at 59–61°C and 44–46°C, respectively (Kaksonen et al. 2007, 2008). Using PCR–DGGE and real-time PCR, bacterial communities in an anaerobic reactor for treating carbazole-containing wastewater have been studied. At 70°C, dominant bacterial representatives of *Pseudomonas* sp., *Comamonas* sp., and *Diaphorobacter* sp. have been identified (Tan and Guodong 2010).

In an anaerobic digester for the treatment of wastewater, small sludge granules begin to form whose surface area is covered in aggregates of bacteria. The flow conditions create a selective environment in which only those microorganisms, which are capable of attaching to each other, survive and proliferate. Eventually the aggregates form into dense compact biofilms referred to as granules. Hence the name granular sludge is given to the sludge derived from such anaerobic digester. Microorganisms of different physiological varieties have also been isolated from sewage sludge (Shooner et al. 1996; Sekiguchi et al. 2000, 2003, 2008; Plugge et al. 2002; Manaia et al. 2003; Diaz et al. 2007). A syntrophic fatty-acid-oxidizing anaerobe, *Syntrophothermus lipocalidus*, has been isolated from granular sludge in a thermophilic upflow anaerobic sludge blanket (UASB) reactor. Crotonate is the only substrate that allows the strain to grow in pure culture. In a coculture with the

thermophilic, hydrogenotrophic *Methanothermobacter thermautotrophicus* strain DeltaH, the isolate syntrophically oxidizes saturated fatty acids (Sekiguchi et al. 2000). Another anaerobic syntrophic thermophile *Pelotomaculum thermopropionicum* has been described by Imachi et al. 2002. This propionate-oxidizing bacterium grows in coculture with *Methanothermobacter thermautotrophicus* strain DeltaH on a variety of substrates. Another thermophilic syntroph, namely, strain TPO, has been isolated from granular sludge from a laboratory-scale upflow anaerobic sludge bed reactor. This strain grows fermentatively as a pure culture in the presence of pyruvate, benzoate, fumarate,  $H_2CO_2$ , pyruvate, and lactate. Comparison of 16S rDNA sequences relates this strain to *Desulfotomaculum thermobenzoicum* (98%) and *Desulfotomaculum thermoacetoxidans* (98%) (Plugge et al. 2002). Three anaerobic, syntrophic primary alcohol- and lactate-degrading thermophilic strains of *Tepidanaerobacter syntrophicus* have also been described from municipal solid waste and sewage sludge digesters (Sekiguchi et al. 2006). A thermophilic multicellular filamentous chemoorganotrophic anaerobe, *Anaerolinea thermophila*, have been isolated from thermophilic granular sludge in an upflow anaerobic sludge blanket reactor treating fried soybean-curd manufacturing wastewater (Sekiguchi et al. 2003). From an upflow anaerobic sludge bed reactor treating brewery wastewater, a moderately thermophilic bacterium, *Aminiphilus circumscriptus*, has been reported (Diaz et al. 2007). Jiang et al. 2005 isolated a thermophilic obligately methylotrophic and methanogenic archaeon *Methanomethylovorans thermophila* that has been isolated from an upflow anaerobic sludge blanket reactor. Three obligately anaerobic, thermophilic, sulfate-reducing bacteria *Thermodesulfovibrio islandicus*, *Thermodesulfovibrio aggregans*, and *Thermodesulfovibrio thiophilus* have also been described from methanogenic sludges derived from the waste and wastewater treatment plants operating at 55°C (Sekiguchi et al. 2008).

There have been relatively few reports on the description of aerobes from wastewater and sewage sludge. On studying the microbial diversity of thermophilic aerobic suspended carrier biofilm process (SCBP) for the on-site treatment of pulp and paper mill whitewater lining by LH-PCR (length heterogeneity) amplified 16S ribosomal DNA, most prominent representatives belonging to  $\beta$ -Proteobacteria, Cytophaga/Flexibacter/Bacteroides group, and  $\gamma$ -Proteobacteria have been described (Tirola et al. 2003). A strictly aerobic facultative autotroph *Thiobacillus thermosulfatus* has been isolated from sewage sludge samples enriched with elemental sulfur. This thermophilic organism can reduce elemental sulfur and can grow autotrophically in a temperature range of 34–65°C with optimum growth occurring at pH 5.2–5.6 and 50–52.5°C (Shooner et al. 1996). Manaia et al. (2003) described an aerobic moderately thermophilic bacterium, *Tepidiphilus margaritifera*, from water-treatment sludge aerobic digester operating at temperatures around 60°C. They described another thermophilic aerobe, *Caenibacterium thermophilum*, from digester of a municipal sludge.

There are few reports on the isolation of thermophiles from other waste and waste treatment sources. Nakamura et al. (2004) reported the characterization of a moderately thermophilic and alkaliphilic bacterium from a semicontinuous decomposing system of kitchen refuse. This novel bacterium, *Cerasibacillus quisquiliarum*, shows optimum temperature of growth at 50°C. A thermotolerant iron-oxidizing

acidophilic aerobe, *Sulfobacillus thermotolerans*, has been isolated from a gold-recovery plant, Siberia (Bogdanova et al. 2006). From a methanogenic bioreactor treating artificial solid waste, an anaerobic, moderate thermophile (temperature optima 55–58°C), *Lutispora thermophila*, has been isolated (Shiratori et al. 2008).

### 1.2.2.3 Self-Heated Compost Piles

Composting is a self-heating, aerobic, bio-decomposition of organic waste materials. Composting is done either on the ground (static piles or aerated piles) or can be accomplished more rapidly using a bioreactor. Under favorable conditions, self-heating static piles typically raise the temperature inside a compost pile to 55–60°C or above within few days. After a few days, there is a gradual decline in the temperature. Aerated pile process achieves faster composting rates through improved aeration by the perforated pipes. Inside the pile, temperature rises to the self-limiting levels of 70–80°C. Composting in a bioreactor is accomplished more rapidly, and the temperature of the compost piles rises to 76–78°C.

#### Diversity of Thermophilic Microorganisms in Compost

Microorganisms have been shown to appear in succession during the composting process. Composting is initiated by mesophilic heterotrophs. But as the temperature rises, these are replaced by the thermophilic forms. Above 60°C, thermophilic fungi become active, and further microbial heat production is due solely to bacteria and actinomycetes. Diversity of microflora in compost depends on different factors like age, substrate materials used, and temperature. Many thermophiles, which are known to be prominent in every composting process, are *Bacillus stearothersophilus*, *Thermomonospora*, *Thermoactinomyces*, and *Clostridium thermocellum*. Other bacterial representatives include both gram-negative as well as gram-positive species. Fujio and Kume 1991 isolated thermophilic strains of *Bacillus stearothersophilus* and *Thermus* sp. from a sewage sludge compost under aerobic conditions at 60°C. In a study based on cultivation as well as restriction enzyme analysis of a clone library of bacterial 16S rRNA genes, Blanc et al. (1999) revealed the presence of *Thermus thermophilus*, *Bacillus* spp., and *Hydrogenobacter* spp. in thermogenic composts. Several novel microorganisms have been isolated from the different types of composts. An aerobic thermophilic bacterium, *Geobacillus toebii*, with temperature optimum of 60°C, isolated from hay compost in Korea, has been reported by Sung et al. (2002). Other novel aerobic thermophilic bacilli, *Tuberibacillus calidus* and *Paenibacillus humicus*, have been isolated from compost pile and poultry litter compost, respectively (Hatayama et al. 2006; Vaz-Moreira et al. 2007). Several gram-negative bacteria have also been isolated from composts. *Luteimonas composti* is a yellow-pigmented gram-negative bacterium isolated from food waste compost, Kinmen County, Taiwan (Young et al. 2007). *Thermobacillus composti*, a moderately thermophilic bacterium, has been isolated from compost bioreactor (Watanabe et al. 2007). Weon et al. 2007 isolated two gram-negative thermophilic

members of *Ureibacillus*, *Ureibacillus composti*, and *Ureibacillus thermophilus* from a compost sample from a compost facility in Ichon, Korea. Two extremely thermophilic bacteria with temperature optimum of 78°C, *Calditerricola satsumensis* and *Calditerricola yamamurae*, have been described from high-temperature compost by Moriya et al. 2011. Thermophilic archaeal methanogens have also been found to dominate during composting. Derikx et al. (1989) identified strains of *Methanobacterium thermoautotrophicum*, which grow only on H<sub>2</sub> and CO<sub>2</sub> as energy and carbon source and do not require complex factors for growth. The 16S rDNA-based phylogenetic surveys of compost revealed an increase of archaeal diversity with compost maturation. 16S rDNA clones related to *Methanothermobacter* spp. and *Methanosarcina thermophila* have been found (Thummes et al. 2007).

Being traditionally studied by the culture-based methods, molecular tools like fatty acid profiling, PCR-based single-strand conformation polymorphism (PCR-SSCP) and denaturing gradient gel electrophoresis (PCR-DGGE), and 16S rRNA gene analysis are now in common use for studying the composting process. Several cloned 16S rDNA sequences from high-temperature (64–84°C) kitchen and garden waste composts have been found to be related to *Thermus* species (Blanc et al. 1999; Beffa et al. 1996). Other rDNA sequences from that study closely resembled sequences from bacteria that have not been previously associated with hot compost, including *Saccharococcus thermophilus* and *Rhodothermus marinus* (Blanc et al. 1999). Several composted materials have been found to contain ammonia oxidizer-like 16S rDNA sequences from β-Proteobacteria, using DGGE and a competitive PCR method (Kowalchuk et al. 1999). Using PCR-amplified small-subunit rRNA genes (SSU rDNA), the diversity and succession of microbial communities during composting has been studied. DNA sequencing of these molecular isolates shows similarities with gram-positive bacteria with a low and high G+C DNA content and to the SSU rDNA of γ-Proteobacteria. The amplified 18S rRNA gene sequence relates to the regions of *Candida krusei* and *Candida tropicalis* (Peters et al. 2000). Dees and Ghiorse (2001) detected high diversity of uncultivated bacteria associated with synthetic food waste compost using ARDRA. Phylogenetic analysis using sequences of 16S rDNA also revealed the presence of *Aneurinibacillus* and *Brevibacillus*, which are not commonly associated with hot compost. *Bacteroides* have been found to be the most dominant bacteria in cow manure composts using DGGE (Green et al. 2004). Xiao et al. (2011) found the presence of actinomycetes using DGGE and quantitative PCR (qPCR), in a continuous thermophilic composting process at temperatures higher than 50°C (Xiao et al. 2011).

Fungi have been cultured from all four phases of the compost cycle but appear most prevalent during the initial and middle mesophilic phases (De Bertoldi et al. 1983; Ryckeboer et al. 2003a, b). The most dominant fungi cultured from the mesophilic phases are species in the genera *Acremonium*, *Alternaria*, *Aspergillus*, *Chaetomium*, *Cladosporium*, *Emericella*, *Fusarium*, *Geotrichum*, *Mortierella*, *Mucor*, *Penicillium*, *Pseudallescheria*, *Scopulariopsis*, and *Trichoderma*. *Absidia*, *Aspergillus*, *Chaetomium*, *Coprinus*, *Mucor*, *Paecilomyces*, *Penicillium*, *Rhizomucor*, *Scytalidium*, and *Thermomyces* are thermotolerant genera that have been isolated at higher temperatures (Waksman et al. 1939; Ghazifard et al. 2001; Vijay et al. 2002; Ryckeboer et al. 2003a, b; Anastasi et al. 2005). Work on thermophilic fungi in

composts has been reviewed by Cooney and Emerson (1964), Kane and Mullins (1973), and Gray (1970). Important thermophilic fungi include *Geotrichum candidum*, *Aspergillus fumigatus*, *Mucor pusillus*, *Chaetomium thermophile*, *Thermoascus aurantiacus*, and *Torula thermophila* (Tansey and Brock 1978). In a recent culture-independent study using DGGE and analysis of rDNA clone libraries, sequences affiliated to *Candida tropicalis*, *Candida krusei*, *Cercophora*, *Neurospora*, and different Basidiomycetes have been reported from organic municipal waste compost (Bonito et al. 2010). Few fungi like *Thermomucor* (Subrahmanyam et al. 1977) and *Myceliophthora thermophila* (Sen et al. 1980) were isolated from municipal waste compost in India.

### 1.2.3 Other Thermophilic Habitats

Few organisms have also been isolated from the other man-made/natural thermophilic habitats apart from those that are described above. A xylanolytic enzyme-producing bacterial strain of *G. thermoleovorans* was isolated from a pulp sample from Century Paper Mills, Uttaranchal (India) (Sharma et al. 2007). A novel triangular-shaped thermophilic and sulfate-reducing archaeon *Archaeoglobus sulfaticallidus* sp. nov. was isolated from black rust formed on the steel surface of a borehole observatory (CORK 1026B) retrieved during IODP Expedition 301 on the eastern flank of Juan de Fuca Ridge, Eastern Pacific Ocean. *Archaeoglobus sulfaticallidus* sp. nov., a novel thermophilic and facultatively lithoautotrophic sulfate reducer isolated from black rust, exposed to hot ridge flank crustal fluids (Steinsbu et al. 2010). An obligately anaerobic extreme thermophilic bacterium, *Caldicoprobacter oshimai* gen. nov., sp. nov., was isolated from sheep feces. This bacterium grows optimally at 70°C and pH 7.2 (Yokoyama et al. 2010). Besides these, several thermophilic fungi have been isolated from habitats like stored peat (Kuster and Locci 1964), birds' nests (Satyanarayana et al. 1977; Tansey 1973), and stored oil palm kernels (Eggins and Coursey 1968). Subrahmanyam (1999) and Johri and Satyanaryana (1986) also reviewed the ecology of thermophilic fungi from different thermophilic habitats like hay, paddy straw, coal spoil tips, stored grains, coal mine soils, and wood chip piles.

## 1.3 Adaptations in Thermophiles

Cellular structure and activities are affected by various factors including temperature. For any microbe to grow at high temperature, its major components, including proteins nucleic acids and lipids, must be able to resist heat. Hence, thermophiles have accumulated various adaptations that allow them to prevail and thrive at high temperatures.

The thermostability of enzymes from thermophiles can be as high as up to 140°C (Adams and Kelly 1995). Structural studies of several thermostable proteins have



shown that some features are highly correlated with thermostability (Ladenstein and Antranikian 1998). A hydrophobic core helps exclude solvent from the internal regions of the protein, making it more resistant to unfolding. A significant increase in the proportion of NTN codons that encodes for nonpolar, hydrophobic amino acids (isoleucine, leucine, methionine, phenylalanine, and valine) has been found to be correlated with increasing optimal growth temperature for most of the bacteria and archaea. This indicates use of hydrophobicity for stabilizing proteins at high temperatures (Lieph et al. 2006). A small surface-to-volume ratio probably improves stability by conferring a compact form on the protein. A reduction in the glycine content of thermostable proteins introduces rigidity, and this, along with the extensive ionic interactions that form a network over the surface of the molecule, helps the compacted protein resist unfolding at high temperature (Ladenstein and Antranikian 1998). Thermophilic proteins show higher proportion of thermophilic amino acids (e.g., proline residues with fewer degrees of freedom). A higher content of arginine and a reduction in the total number of thermally unstable residues, such as Cys, Lys, Met, Asn, and Gln (Cicicopol et al. 1994), have been reported for thermostable proteins. Protein stability may also be assisted by accumulation of intracellular potassium and solutes such as 2,3-diphosphoglycerate.

Proteins have evolved ways to maintain protein integrity and function at high temperatures. These include increasing ion-pair content, or a change from monomeric to oligomeric structure (Cicicopol et al. 1994); additional networks of hydrogen bonds (Jaenicke and Bohm 1998); an increase in disulfide bond formation (Beeby et al. 2005), decreasing the length of surface loops that connect elements of secondary structure (Thompson and Eisenberg 1999); and exchange of amino acids to increase helix propensity of residues in  $\alpha$ -helices. Presence of noncovalent, ionic bonds called salt bridges on a protein's surface likely has shown to play a major role in maintaining the biologically active structure of proteins (Das and Gerstein 2000).

In addition to these factors, some assisting proteins, such as molecular chaperonins, also facilitate protein thermostability. Chaperonins (heat shock proteins) function to refold partially denatured proteins. Hyperthermophilic archaea produces special classes of chaperonins that function only at the highest growth temperatures. A chaperonin known as thermosome (Andr a et al. 1998) has been characterized from thermophiles *Methanopyrus kandleri*, *Pyrococcus abyssi*, and *Pyrodictium occultum*. This complex is thought to bind heat-denatured proteins, prevent their aggregation, refold them into their active form (Andr a et al. 1998), and help cells survive, even at temperatures above their maximal growth temperature. For example, the cells of *Pyrococcus abyssi* can remain viable even after 1-h treatment in autoclave (121°C). Thus, the upper temperature limit at which many hyperthermophiles can survive is higher due to chaperonin activity than that the upper temperature at which they can grow.

Several factors may combine to afford heat stability to DNA in thermophiles including high levels of  $K^+$  (Marguet and Forterre 1998), reverse DNA gyrase (van der Oost et al. 1998; Forterre et al. 1996), and histone or other DNA-binding proteins (Pereira and Reeve 1998). Cytoplasm of the hyperthermophilic methanogens *Methanopyrus* contains molar levels of potassium and cyclic 2,3-diphosphoglycerate.



This solute prevents depurination or depyrimidination of DNA, which causes mutation (Marguet and Forterre 1998) at high temperatures.

Positive supercoiling of DNA may be an important factor stabilizing DNA to high temperatures. All hyperthermophiles produce a unique protein called reverse gyrase (van der Oost et al. 1998; Forterre et al. 1996). This is a type I DNA topoisomerase. It has been shown to catalyze the positive supercoiling of closed circular DNA. For various reasons, in particular, its higher linking number, positively supercoiled DNA is more resistant to thermal denaturation than is negatively supercoiled DNA (Forterre et al. 1996). Moreover, monovalent and divalent salts enhance the stability of nucleic acids because these salts screen the negative charges of the phosphate groups and because KCl and  $MgCl_2$  protect the DNA from depurination and hydrolysis (Marguet and Forterre 1998). The G–C pair of nucleic acids is more thermostable than the A–T or A–U pairs because of the additional hydrogen bond (Galtier et al. 1999). But elevated G and C ratios are not found in the genomes of thermophilic prokaryotes, although thermostability has been correlated with increase in G–C pairs of their SSU rRNA and transfer RNAs (Galtier and Lobry 1997).

In addition, other proteins in hyperthermophiles also function to maintain the integrity of the DNA duplex. Several Euryarchaeotes contain histone-like proteins, which resemble to the core histones of Eukaryotes in structure and function (Sandman et al. 1998; Pereira and Reeve 1998). They wind and compact DNA into nucleosome-like structures (Pereira et al. 1997) that maintain DNA in a double-stranded form at high temperatures (Soares et al. 2008). Small DNA-binding proteins like Sac7d in the *Sulfolobus acidocaldarius* bind to the minor groove of DNA nonspecifically and increase the melting temperature of DNA by some 40°C (Robinson et al. 1998). Archaeal histones from thermophilic methanogens like *Methanothermus fervidus* and halophiles like *Halobacterium* have been well studied.

Polyamines also play a role in DNA stability and stability of other macromolecules. These molecules (e.g., putrescine and spermidine) together with  $Mg^{2+}$  function to stabilize RNA and DNA. In thermophilic archaea such as *Sulfolobus*, polyamines help stabilize ribosomes, thereby facilitating protein synthesis at high temperatures.

High temperature also increases the fluidity of membranes. To maintain optimal membrane fluidity, the cell must adjust the membrane composition, that is, the amount and type of lipids. So, the membrane lipids of thermophiles contain more saturated and straight-chain fatty acids than mesophiles. This allows thermophiles to grow at higher temperatures by providing the right degree of fluidity needed for membrane function. Many archaeal species contain a paracrystalline surface layer (S-layer) with protein or glycoprotein that functions as an external protective barrier.

## 1.4 Future Perspectives and Conclusions

Natural and man-made habitats with elevated temperatures are known to exist throughout the globe. Most of the natural habitats are high-temperature zones where the source of heat is geothermal in origin. In man-made habitats, heat generation is

due to self-heating or solar heating, and temperature in these habitats can reach up to 70–80°C. An unanticipated phylogenetic and physiological diversity of thermophiles exists in the different thermophilic habitats. The representatives of all the microbial groups have been described from these habitats through cultivation-dependent as well as cultivation-independent approaches. Most of the extreme thermophiles and hyperthermophiles have been revealed from natural habitats, which include bacteria and archaea. Moderately thermophilic members include prokaryotes as well as eukaryotic fungal and algal species, which are isolated from natural as well as man-made habitats. In these habitats, thermophiles are either primary producers or consumers of organic matter. Energy conservation in primary producers occurs by anaerobic and aerobic types of respiration. Consumers gain energy either by anaerobic or aerobic types of respiration or by fermentation.

Cultivation has been a prerequisite for understanding their physiology and role in high-temperature ecosystems. Besides those isolated by culture-dependent approaches, several uncultured organisms are still to be cultivated. To cultivate these uncultivated organisms is a critical challenge in the future. Understanding their physiology through cultivation shall provide an insight into novel enzymes and metabolic products produced by them. These may further be suitable for use in novel biotechnological processes.

The understanding of genomes of the thermophilic organisms has provided valuable information on their gene sequences and high-resolution 3-D structures of their proteins. Despite the availability of such vast amount of information from thermophiles, the biophysical basis of their survival at high temperatures remains a debatable topic.

Another important aspect that needs to be considered is the upper temperature limit of life. The currently known upper limit of life is 122°C. It, however, appears improbable that this represents the endpoint of the search of hyperthermophilic organisms. The study of this aspect is important as the upper temperature limit for life is a key parameter for delimiting when and where life might have evolved on the hot Earth in the geological past, the depth to which life exists in the Earth's subsurface, and the potential for life in hot extraterrestrial environments.

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