Chapter 5
Metagenomics of Hyperthermophilic Environments: Biodiversity and Biotechnology

Andrea Strazzulli, Roberta Iacono, Rosa Giglio, Marco Moracci and Beatrice Cobucci-Ponzano

Abstract The field of thermophilic microbiology was born in the late 1970s with the pioneering work of Brock (Thermophiles biodiversity, ecology, and evolution. Springer, Boston, pp. 1–9, 2001) and dramatically expanded through the '80s with the isolation of hyperthermophiles by Stetter (FEMS Microbiol Rev 18:149–158, 1996). The development of SSU rRNA phylogenetics revealed the complexity and diversity of prokaryotic phylotypes on biotopes widely differing in extreme conditions (e.g. spanning gradients of pH between 0 and 10 and temperatures from 60 °C to over 120 °C, respectively). Sites of volcanic activity all over the Earth's surface and under the sea provide a variety of different environments for extremophilic microorganisms. Hot springs populated by hyperthermophiles (T_{opt} > 65 °C), the majority of which belonging to the domain of Archaea, are very diverse and some of them show combinations of other extreme conditions, for example, acidic, alkaline, high pressure, and high concentrations of salts and heavy metals (Cowan et al. in Curr Opin Microbiol 25:97–102, 2015). Archaea inhabiting hot springs are considered to be the closest living descendants of the earliest living forms on Earth and their study provide insights into the origin and evolution of life (Woese et al. in Proc Natl Acad Sci USA 87:4576–4579, 1990; Olsen et al. in J Bacteriol 176:1–6, 1994). As with all studies of environmental microbiology, our understanding of the function of (hyper)thermophilic microbial consortia has lagged substantially behind. However, recent advances in 'omics' technologies, particularly within a system biology context, have made significant progresses into the prediction of in situ functionality (Cowan et al. in Curr Opin Microbiol 25:97–102, 2015). Most extremophilic microorganisms are recalcitrant to cultivation-based approaches (Amann et al. in Microbiol Rev 59:143–69, 1995; Lorenz et al. in Curr Opin Biotechnol 13:572–577, 2002); therefore, culture-independent metagenomic strategies are promising approaches to assess the phylogenetic composition and functional potential of microbial communities living in extreme environments (López-López et al. in Life 3:308–320, 2013). In addition, these approaches

A. Strazzulli · R. Iacono · R. Giglio · M. Moracci · B. Cobucci-Ponzano (✉)
Institute of Biosciences and Bioresources, National Research Council of Italy,
Via P. Castellino 111, 80131 Naples, Italy
e-mail: beatrice.cobucciponzano@ibbr.cnr.it

C. Chénard and F.M. Lauro (eds.), *Microbial Ecology of Extreme Environments*,
DOI 10.1007/978-3-319-51686-8_5

implement tremendously the access to enzymes from (hyper)thermophilic microorganisms that have important potential applications in several biotechnological processes. We report here on the state-of-the-art of the metagenomic surveys of different hot springs (T > 65 °C) (Table 5.1) and on the recent advance in the discovery of new hyperthermostable biocatalysts of biotechnological interest from metagenomic studies of these extreme environments.

5.1 Yellowstone National Park

The Yellowstone geothermal complex in the Yellowstone Caldera area is one of the most studied geothermal locations that includes more then 10,000 thermal sites such as geysers, mud pools, hot springs and vents showing a broad range in pH, temperature and geochemical properties. All of these conditions make of the entire geothermal area a natural laboratory for the study of the hyperthermophilic microbial communities since 1953 shedding light on their evolution, metabolic potential and adaptation to high-temperature environments (Marsh and Larsen 1953).

In 2005, Meyer-Dombard and co-workers reported a detailed environmental and microbiological survey of three different hot springs in Yellowstone National Park (YNP): Obsidian Pool (ObP) (80 °C, pH 6.5), Sylvan Spring (SSp) (81 °C, pH ∼ 5.5), and Bison Pool (BP) (83 °C, pH ∼ 8.0) (Table 5.1) (Meyer-Dombard et al. 2005). By the analysis of 16S rRNA genes, they reported that Thermocrinis, followed by Geothermobacterium and different proteobacteria, dominate the bacterial community in ObP and BP. In addition, the archaeal community was mostly composed by different groups of uncultured Crenarchaeota in ObP, while several members belonging to family Desulfurococcaceae were found in BP. By contrast, in SSp they identified *Hydrogenothermus* as the most abundant bacterial genus and, among the archaea, observed the dominance of the families Desulfurococcaceae and Thermoproteaceae (Meyer-Dombard et al. 2005).

A first wide metagenomic survey of the microbial species in the YNP was addressed by Inskeep and co-workers in 2010 (Inskeep et al. 2010) aiming to identify the predominant microbial populations of five geochemically different high-temperature environments, their metabolic potentials and the genes probably related to the different geochemical conditions across these sites (Table 5.1). Crater Hills (CH) (75 °C, pH 2.5) is a turbid acidic pool with dissolved oxygen lower than 3 μM, low concentrations of dissolved H_2S, H_2 and CH_4 and containing particles in suspensions basically composed by elemental S and SiO_2. The acidic sample collected in Norris Geyser Basin (NGB) (65 °C, pH 3.0) from an oxygenated outflow channel is rich in FeIII-oxides and showed dissolved O_2 concentration in a range of 30–100 μM. Previous studies report that NGB, as well as CH, contain significant numbers of Crenarchaea of the order Sulfolobales (Inskeep et al. 2005; Young et al. 2005; Kozubal et al. 2008). Joseph's Coat (JCHS), Calcite (CS), and Mammoth Hot Springs (MHS) are all sulfidic hot springs with pH > 6.1 and lower concentration of dissolved O_2, which differ geochemically from one another and show significant

Table 5.1 State-of-the-art of the metagenomic surveys of different hot springs

Abbreviation	Location	Coordinates	Temperature (°C)	pH	Dominant microorganisms	References
ObP	Yellowstone National Park, USA	44.60302 N, 110.86519 W	80	6.5	Aquificales (*Thermocrinis*) Uncultured Crenarchaeota	Meyer-Dombard et al. (2005)
SSp		44.69886 N, 110.76827 W	81	~5.5	Aquificales (*Hydrogenothermus*) Desulfurococcaceae–Thermoproteaceae	
BP		44.56969 N, 110.86519 W	83	~8.0	Aquificales (*Thermocrinis*) Uncultured Crenarchaeota	
DS		42.46313 N, 110.82983 W	68–72	3.1	Aquificales (*Hydrogenobaculum*)	Inskeep et al. (2013b), Beam et al. (2014), Takacs-Vesbach et al. (2013)
OSP_14		44.73304 N, 110.70899 W	72–74	3.5	Aquificales (*Hydrogenobaculum*)	Takacs-Vesbach et al. (2013), Inskeep et al. (2010), (2013b)
MHS		44.96942 N, 110.70983 W	70–72	6.5	Aquificales (*Sulfurihydrogenibium*)	Inskeep et al. (2010), (2013b), Takacs-Vesbach et al. (2013)
CS		44.90485 N, 110.40402 W	74–76	7.8	Aquificales (*Sulfurihydrogenibium*)	Inskeep et al. (2013a, b), Takacs-Vesbach et al. (2013)
OS		44.53401 N, 110.79781 W	80–82	7.9	Aquificales (*Thermocrinis*)	Schoenfeld et al. (2008), Pride and Schoenfeld (2008), Takacs-Vesbach et al. (2013)
BCH		44.2829 N, 110.9053 W	80–82	7.8	Aquificales (*Thermocrinis*)	Inskeep et al. (2010), (2013b), Takacs-Vesbach et al. (2013)
CH		44.65328 N, 110.48474 W	76	2.6	Crenarchaeota (*Sulfolobales*)	Inskeep et al. (2013a, b), Menzel et al. (2015)

(continued)

Table 5.1 (continued)

Abbreviation	Location	Coordinates	Temperature (°C)	pH	Dominant microorganisms	References
NL		44.75232 N,	88–92	3.0–4.0	Crenarchaeota (*Sulfolobales*)	Inskeep et al. (2013a), Menzel et al. (2015) Bolduc et al. (2012)
MG		44.72429 N, 110.70561 W	70–80	4.0	Crenarchaeota (*Desulfurococcales*, *Thermoproteales*)	Inskeep et al. (2013a, b)
CIS		44.72308 N, 110.70402 W	78–80	4.4	Crenarchaeota (*Desulfurococcales*, *Thermoproteales*)	Inskeep et al. (2010), (2013a, b)
JCHS		44.73916 N, 110.32449 W	80	6.1	Crenarchaeota (*Desulfurococcales*, *Thermoproteales*)	Inskeep et al. (2013a, b)
WS		44.76489 N. 110.43035″ W	76	6.4	Crenarchaeota Aquificales	Inskeep et al. (2013a, b)
OSP_8		44.73304 N, 110.70899 W	72	3.4	Crenarchaeota Mixed Novel Archaeal	Inskeep et al. (2013a, b)
NGB		44.7315 N, 110.71136 W	65	3.0	Crenarchaeota (*Sulfolobales*)	Inskeep et al. (2010)
Is2-5S	Iceland	64.03167N, 21.1966 W	85–90	5.0	Aquificales (*Thermocrinis*) Proteo bacteria	Menzel et al. (2015)
Is3-13		63.90416 N, 22.05805 W	90	3.5–4.0		
Kam37	Kamchatka	54.50 N, 159.97 E	81–85	5.5–7.4	Aquificales Euryarchaeota Crenarchaeota	Eme et al. (2013), Wemheuer et al. (2013)
Not assigned		52.453 N, 158.195 E	70	3.5–4.0	Miscellaneous Crcnarchacotic Group	Wemheuer et al. (2013)

(continued)

Table 5.1 (continued)

Abbreviation	Location	Coordinates	Temperature (°C)	pH	Dominant microorganisms	References
					Enterobacteriaceae Miscellaneous Crenarchaeotic Group	
AI	Furnas Valley, Azores	37.7725 N, 25.303889 W	51	3.0	Proteo bacteria	Sahm et al. (2013)
AII			84	2.5–3.0	*Not reported*	
AIII			85	8.0	*Not reported*	
AIV			92	8.0	Aquificae, Dictyoglomi	
BII			65	7.0	*Not reported*	
BIII			70	7.0	*Not reported*	
CII			76	8.0	*Not reported*	
SK	Perak, Malaysia	3.99663 N, 101.39310 E	80	8.0	Firmicutes	Chan et al. (2015)
Dgg	Hot Springs of Rehai region in Tengchong, China	24.95344 N. 98,43780 E	84.5	7.2	Aquificae	Hou et al. (2013)
Drty-l		24.95396 N, E98.43829E	85.1	2.6	Crenarchaeota	
GmqS			93	9.3	Aquificae	
GmqC		24.57060 N, 98.43615 E	89	9.4	Aquificae (water) Crenarchaeota (sediment)	
GmqP			82.5	9.3	Aquificae Aquificae (water)	
JmqL		24,95112 N, 98 43600 E	93.6	9.2	Crenarchaeota (sediment)	

(continued)

Table 5.1 (continued)

Abbreviation	Location	Coordinates	Temperature (°C)	pH	Dominant microorganisms	References
JmqR		24.95115 N, 98 43596 E	83.2	9.4	Aquificae	
Zzq			89.1	4.8	Crenarchaeota	
HtjL		24.95089 N, 98 43664 E	90	8.1	Crenarchaeota Crenarchaeota	
HtjR			92.3	8.0	Aquificae	
SrbzU		24.95002 N, 98.43728 E	79.8	8.0	Aquificae	
SrbzD			78.2	8.3	Aquificae	
Gxs	Hot Springs of Ruidian region in Tengchong, China	25.44012 N, 98.44081 E	73.8	7.3	Bacteria	Hou et al. (2013)
Jz		23.44138 N 98.46004 E	81.6	6.7	Bacteria Crenarchaeota	
CPc	Taupo volcanic zone, New Zealand	38.3591 S, 176.36990 E	75	5.8	Aquificales (*Sulfurihydrogenibium*)	Hug et al. (2014)
CPp			75	5.5		
CPr			68	5.5		
AP			45	6.9		
It6	Phlegraean Fields, Italy	40.82690 N. 14.13914 E	76	3.0	Proteobacteria (*Acidithiobacillus*)	Menzel et al. (2015)
It3		40.82920 N, 14,14712 E	86	5.5	Crenarchaeota (*Acidianus*)	

differences in the microbial communities. The sediment sampled at JCHS (80 °C, pH 6.1) is rich of sulfides and elemental S while the aqueous phase contains high concentration of CH_4, H_2, NH_4^+, arsenite and thiosulfate showing several reduced chemical species that could serve as electron donors for the chemolithotrophic metabolism with low concentration of O_2. CS (75 °C, pH 7.8) and MHS (71 °C, pH 6.6) samples were collected from high-velocity, highly-sulfidic outflow channels and have been reported to be dominated by microorganisms of the order Aquificales (Fouke et al. 2000, 2003; Reysenbach et al. 2005). Using binning and fragment recruitment approaches, Inskeep and collaborators observed that high-temperature springs with acidic pH were dominated by Archaea with distantly related organisms whose genomes have been sequenced and that can be assigned to the orders Sulfolobales (CH and NGB) and Thermoproteales (JCHS). Moreover, while a significant number of contigs were assigned to novel populations of Desulforococcales in all the three archaeal-dominated sites, just a modest number of sequences were assigned to Sulfolobales in JCHS. These results suggest that the members belonging to the class Thermoprotei are common in the archaeal communities of YNP and that their relative abundance is modulated by differences in pH and/or by the concentration of dissolved O_2. Conversely, Bacteria belonging to the order Aquificales dominated the two microbial communities inhabiting CS and MHS with pH > 6.0. In particular, about 90% of the reads found in MHS showed a nucleotide identity greater than 90% with *Sulfurihydrogenibium* sp. Y03AOP1 isolated in Obsidian Pool (Reysenbach et al. 2009) while the reads obtained from CS were assigned with high identity to *Thermus aquaticus* and Sulfurihydrogenibium yellowstonensis.

The more recently presented "YNP metagenome project" (Inskeep et al. 2013b), comparing metagenomes and geophysical parameters of 20 different geothermal sites in the YNP, represents one of the most complete studies of the communities populating high-temperature environments. Thirteen of them with T > 65 °C have been grouped in two different ecosystem types based on primary environmental factors, such as pH, temperature, presence of dissolved sulfide and elemental S, and additional physiographic parameters. The first ecosystem groups six sites populated by Aquificales-rich "filamentous-streamer" communities (Table 5.1): Dragon Spring (DS; 68–72 °C, pH 3.1); 100 Sping Plan (OSP_14; 72–74 °C, pH 3.5), Octopus Spring (OS; 74–76 °C, pH 7.9) and Bechler Spring (BCH; 80–82 °C, pH 7.8) together with MHS and CS described above (Takacs-Vesbach et al. 2013; Inskeep et al. 2013b). Takacs-vesbach and co-workers, by using a phylogenetic and functional analysis of the metagenomic samples from the "filamentous-streamer" communities, reported three lineages of Aquificales with a potential metabolism related mainly to pH and sulfide and/or elemental sulfur. DS and OSP_14, showing low-pH (pH 3.1–3.5) and high-sulfide concentration, contained *Hydrogenobaculum* spp., whereas higher-pH sites were dominated by *Sulfurihydrogenibium* spp., as in MHS and CS, or *Thermocrinis*-like populations, as in OS and BCH. Among these sites, CS is the only one in YNP hosting *Thermocrinis*-like population together with *Sulfurihydrogenibium* spp. as expected by previous 16S rRNA surveys that reported only minor overlap in the distribution of different Aquificales species across the

YNP geothermal environments (Reysenbach et al. 2005; Hall et al. 2008; Hamamura et al. 2009). In addition, the authors used an automated phylogenomic inference pipeline for bacterial sequences to detect the number of single-copy genes and to evaluate the sequence heterogeneity within the Aquificales populations. In this way, it was observed that although the community from MHS was dominated by a quite homogeneous population of *Sulfurihydrogenibium* spp., the sites OS, BCH and CS, carrying higher numbers of single-copy genes, showed greater heterogeneity of *Thermocrinis*-like populations in situ. Differently, the acidic sites DS and OSP_14 contained, together with the primary populations *Hydrogenobaculum* spp., different archaeal populations such as *Metallosphaera*-like, Thaumarchaeota and Thermoplasmatales-like, consistently with the analysis reported by Pride and Schoenfeld (2008), Schoenfeld et al. (2008). In particular, although both the higher-pH sites OS and BCH with low-sulfide concentration (<1 μM) contained similar *Thermocrinis*-like Aquificales, Takacs-vesbach reported that the microbial community of OS, which contains at least three additional novel bacterial assemblies related to unclassified 16S rRNA genes described previously (Reysenbach et al. 1994; Blank et al. 2002; Hall et al. 2008), is considerably different if compared to BCH populations. Even though, the inorganic constituents of these two springs were similar enough. On these bases, the authors suggested that the observed differences across these apparently similar sites could be addressed to additional geochemical and geophysical factors, such as the total dissolved organic carbon (DOC) and the amount of solid-phases of carbon, that have not been deeply characterized in their study and that could play a key role in the determination of the influence of the organic constituents on the microbial community structure (Takacs-Vesbach et al. 2013). The linkages among geochemistry, specific phylotypes and their metabolisms have been accounted by the global protein analysis of the "streamer communities" with TIGRFAMs specific to electron transport coupled with principal component analysis (PCA) and hierarchical clustering (HC). Focusing on the geochemistry of the community (in particular pH and sulfide content) Takacs-vesbach indeed observed that the samples from DS (sulfur rich) and OSP_14 (FeIII-oxide rich), although contain a quite similar *Hydrogenobaculum* population, show different archaeal co-communities composed by anaerobic/microaerophilic populations and oxidizing Sulfolobales in DS and OSP_14 sites, respectively. These primary differences among the populations of the sites reflect specific metabolic pathways and are strictly related to the different geochemical habitats. In particular, by the metagenomic sequence of the Aquificales, especially in the *Hydrogenobaculum* and *Sulfurihydrogenibium*-like organisms present in YNP, Takacs-vesbach and colleagues observed a variety of S-oxidation pathways coupled with cbb3-Type C heme-copper oxidases (HCOs) or bd-ubiquinol terminal oxidase complexes, indicating a clear potential for the oxidation of reduced sulfur species. By contrast, they observed that the *Thermocrinis* organism, present in the oxic samples from OS and BCH, contain Type A-HCOs, indicating a functional divergence from the other Aquificales genera, which have copies of both the HCOs type (Takacs-Vesbach et al. 2013).

The second ecosystem in the "YNP metagenome project" includes seven archaeal-dominated sediments (Table 5.1): Nymph Lake (NL; 88 °C, pH 4), Monarch Geyser (MG; 78–80 °C, pH 4.0), Cistern Spring (CIS; 78–80 °C, pH 4.4), Washburn Spring (WS; 76 °C, pH 6.4), 100 Spring Plan (OSP_8; 72 °C, pH 3.4), and including CH and JCHS described above (Inskeep et al. 2013a, b). By following the approach applied for the analysis of the Aquficales-dominated sites, the authors have investigated in detail the thermal sites (70–85 °C and pH range 2.5–6.4) that represent some of the major chemotrophic habitats in YNP (Inskeep et al. 2013a). As for the Aquificales "filamentous-streamer" communities, the archaeal-dominated sites were classified primarily on the base of pH and the presence of dissolved sulfide and elemental sulfur, while the temperature was not taken as a major variable. The abiotic consumption of oxygen by reduced sulfur contributes to the hypoxic conditions observed in CH, NL, MG, JCHS, CIS, and WS. The authors observed that pH was a major factor controlling the distribution of Sulfolobales *versus* Thermoproteales and Desulfurococcales. The combination of acidic pH, reduced sulfur and high-temperature acts as severe constrain on the microbial community diversity; indeed, CH and NL sites with these properties were dominated by only two major Sulfolobales populations. The acidic NL site, together with CH previously described, is a highly turbulent pool that contains suspended particles of elemental sulfur and SiO_2, and low concentration of dissolved sulfide (<5 μM). According to Menzel and collaborators, which analyzed CH and NL sites (Menzel et al. 2015), CH is almost exclusively populated by Archaea with 96.4% of reads (Roche/454 Titanium FLX), which are mostly Sulfolobaceae (85%) made up of *Sulfolobus* and *Acidianus* species. Only 0.2% of the CH reads have been assigned to bacterial species such as *Hydrogenobaculum* spp. and *G. thermoleovorans*. By contrast, the same authors report that NL site shows a higher abundance of Bacteria (12.4% of reads assigned), prevalently Aquificae, and just 58% of reads are assigned to Archaea. As well as CH, the most abundant archaeal family in NL was Sulfolobaceae (32%), followed by Thermoproteales and Acidobales (9 and 2% respectively). In addition, an elevated abundance of archaeal viruses (21% of reads) was found, consistent with those reported previously by Bolduc and co-workers: this was the first time that the presence of putative RNA viral genomes in high-temperature acidic hot springs was found by using a metagenomic approach (Bolduc et al. 2012).

Inskeep et al. reported that also the sulfur sediments from MG, CIS, JCHS, with pH between 4.0 and 6.0, contain a variable amounts of sequence assigned to Sulfolobales populations while the Fe-oxide mats of the sample OSP_8, together with the Fe-oxide "streamer" community in OSP_14, were the only sites with sequences assigned to *M. yellowstonensis*-like populations (Inskeep et al. 2013a). They observed that the main sequence clusters of MG, CIS and JCHS were related to members of the orders Desulfurococcales and Thermoproteales corresponding to *Caldivirga/Vulcanisaeta*-like and *Pyrobaculum*-like organisms. On the basis of nucleotide word frequencies and principal component analysis (NWF PCA), sequence similarity, and GC content, similar populations were also observed in the

non-sulfidic sample OSP_8. This suggests that, although these represent the main community members detected in sulfur-rich sediments from pH 4.0 to 6.0 (Jay et al. 2011) they inhabited also Fe-oxide mats in OSP_8 where sulfide and elemental sulfur are absent. In addition, at last four major archaeal populations were identified in OSP_8: *M. yellowstonensis*, *Vulcanisaeta* spp., *Acidilobus* spp. and a "novel archaeal Group I" (NAG1) (Inskeep et al. 2013a). Regarding the latter, successively Kozubal and colleagues proposed that, representing the most abundant member of the community in OSP_8 with about 20–55% of the total sequence reads, it was a new phylum named Geoarchaeota. The abundance of Geoarchaeota in OSP_8 allowed Kozubal and colleagues to obtain a contig coverage of average ~6x, and a total scaffold length of ~1.7 Mb in only eight scaffolds (Kozubal et al. 2013). Moreover, Inskeep and colleagues, reported that the microbial community of OSP_8 is composed also by several less abundant Archaea including relatives of the Euryarchaeota, Nanoarchaeota, and Crenarchaeota (as other Sulfolobales) (Inskeep et al. 2013a). In addition to these phyla, in the oxic iron mat from OSP_8, Beam and co-workers identified a new candidate phylum of Thaumarchaeota showing a different respiratory machinery respect to the Thaumarchaeota population present in the hypoxic sulfur sediments of DS sample (Beam et al. 2014).

JCHS sites showed evidence of subdominant bacterial populations and do not contain significant numbers of methanogens, while the sediment from WS (80 °C, pH 6.4) contained a significant amounts of sequences related to Bacteria. Inskeep reported that they were more diverse and composed mainly by *Sulfurihydrogenibium* and *Thermodesulfobacteria*-like (~15 and 10% of the assigned reads respectively). Instead, several Thermoproteales populations composed the WS archaeal community, and <1% of the sequences are related to Methanococci and/or Methanosarcinales, which is consistent with the increased abundance of these phylotypes at high pH. In addition, they reported that the PCA of the sequences from these samples, specific to electron transport TIGRFAMs, highlighted the differences in OSP_8 *versus* WS compared to the other sites. They suggest that these differences could be ascribed to the higher abundance of bacterial pathways in WS (*Thermodesulfobacteria*, *Sulfurihydrogenibium*), showing respiratory processes considerably different than the dominant Archaea present in other sites (Inskeep et al. 2013a). Moreover, it was found that WS was the only habitat containing a significant population of Korarchaeota. This result is in line with recent studies on the distribution of korarchaeotal sequences in Kamchatka and YNP showing that these organisms have a narrow pH range of growth (<5.0–7.0) (Auchtung et al. 2011; Miller-Coleman et al. 2012).

The YNP project and the related studies represent a remarkable effort toward understanding the relationship between the microbial community structures and the metabolic potential across the different extremely hot environments in the Yellowstone Caldera. The systematic selection of geochemically distinct sites indeed provides a real guidebook to link specific phylotypes with peculiar physico-chemical properties of the different habitats.

5.1.1 Iceland

Iceland has a high concentration of active volcanoes due to its location on the mid-Atlantic Ridge, a divergent tectonic plate boundary. The island has 30 active volcanic systems most active/volatile. Among these sites, Menzel and coworkers have analyzed through metagenomic approach two sites: Krísuvík and Grensdalur. The total genomic DNA was extracted from both samples (sediment/water), sequenced by Illumina Hiseq and analyzed with MEGAN (Huson and Weber 2013) for estimating species abundances (Table 5.1).

The first site, Krýsuvík (Is3-13), is situated on the Reykjanes peninsula and consists of several geothermal fields, such as Seltún, where solfataras, fumaroles, mud pots and hot springs have formed and the soil is colored in bright yellow, red, and green hues. Is3-13 (90 °C, pH 3.5–4.0) is surrounded by other hot springs and shows very limited access to organic materials. The second analyzed site is the volcano Grensdalur (Is2-5S), with an air distance from Krísuvík of about 45 km. This hot spring (85 °C and pH 5.0) was on a slope of a hill with flow through from other hot springs higher up and the sediment is dark gray and very fine, almost sandy. The surroundings are filled with various organic materials, such as moss and lichen as well as the edges of the spring were encrusted with cyanobacterial or algal mats (Menzel et al. 2015).

From the sample Is3-13 the mapped reads (about 8 millions) were assigned to 79% bacterial and 19.7% archaeal microorganisms. Proteobacteria was the dominant phylum, comprising Gamma- (57%) and Beta-proteobacteria (13%) responsible for nitrogen fixation. Within this phylum, *Acidithiobacillus* (52%) was the most abundant genus. The archaeal community was largely composed of Thermoproteales (13%) hydrogen-sulfur autotrophs, with *Thermoproteus tenax* (12%) being the most abundant species, and Sulfolobales (3%).

In Is2-5S (about 7 millions of mapped reads), Menzel and co-workers reported that almost 33% were assigned to Archaea while 62.1% to Bacteria. These latter were largely comprised of Aquificae (29%), mostly belonging to the species *Thermocrinis albus* (14%) and *Sulfurihydrogenibium azorense* (7%). Crenarchaeota was the dominant archaeal phylum (25%) primarily populated by *Pyrobaculum* genus (13%). The analysis of 16S rRNA predicted from the assembled contigs revealed that many sequences showed > 99% similarity to SILVA database sequences, most of them matching with sequences from species annotated as uncultured (Menzel et al. 2015).

In conclusion, the work of Menzel and colleagues suggests that the community structure is not affected by geographic distance, but by environmental parameters. Indeed, Is2-5S (85 °C/pH 5.0) is similar to the Chinese (65 °C/pH 7.0) and Uzon Caldera in Kamchatka (61–64 °C/pH 5.8–6.0) hot springs and a high overlap between samples can be observed in both archaeal and bacterial components. The sample Is3-13 (90 °C/pH 4.0) is similar to Solfatara volcano from Italy (76 °C/pH 3.0) and both samples have the lowest diversity.

5.1.2 Kamchatka Peninsula

The Kamchatka peninsula, which is located in the Far East of Russia, comprises an area of approximately 472,300 km^2 and is described as the land of fire by its first explorers due to the high density of volcanoes and associated volcanic phenomena. To examine the prokaryotic diversity of the microbial communities in this area, three different metagenomic analyses were performed (Table 5.1) (Eme et al. 2013; Wemheuer et al. 2013). Two samples were from the Central thermal field of Uzon Caldera. The first one is derived from the hot spring referred to as Kam37, characterized by a small bottom opening of 10 × 15 cm with a steady discharge and very little rim overflow, at 85 °C, pH 5.5. The second sample, referred to as Uzon, derived from the same caldera but is 81 °C, pH 7.2–7.4 while the third sample, referred as Mutnovsky, is from a thermal and acidic spring (70 °C, pH 3.5–4.0) at the Mutnovsky volcano.

Environmental DNA for metagenomic analyses was isolated from collected sediment samples by direct cell lysis, and archaeal and bacterial 16S rRNA genes were amplified by PCR and analyzed to assess the prokaryotic community. In particular, Kam37 has been analyzed by constructing a fosmidic metagenomic library and two SSU rRNA gene libraries (one archaeal- and one prokaryotic-specific library). 108 clones obtained with prokaryotic primers and 149 clones from the archaeal-specific library were analyzed. In addition, six selected clones of the fosmid library were sequenced using the 454-pyrosequencing FLX technology, and reads were assembled and annotated. The retrieved 16S rRNA gene sequences were analyzed using QIIME (Caporaso et al. 2010b) to assess the prokaryotic community structure of Uzon and Mutnovsky samples.

The SSU rRNA gene survey of Kam37 suggested that the community was dominated by uncultivated members of the Aquificales, Euryarchaeota, Crenarchaeota, and MCG (Miscellaneous Crenarchaeotic Group), whereas sixteen sequences were similar to Thaumarchaeota, one of the new major archaeal lineages, including putative phyla such as Korarchaeota and Nanoarchaeota. These metagenomic analyses provided the first genomic data from two novel major (hyper)thermophilic archaeal lineages HTC1 and HTC2 (Hot Thaumarchaeota-related Clade 1 and 2, respectively), which represent either an ancient lineage of high-taxonomic rank within Thaumarchaeota. Interestingly, Thaumarcheota, together with Proteobacteria and Thermotogae, dominated also the Uzon and Mutnovsky sites. A study by Meyer-Dombard et al. (2005) investigated the prokaryotic community in three thermal springs in the YNP (the Sylvan Spring, the Bison Pool, and the Obsidian Pool). They have observed that the Sylvan Spring has a low pH of 5.0, whereas the other pools have a rather neutral pH. However, the prokaryotic community structure of this acidic spring was different to that found in the acidic Mutnovsky spring sample. Meyer-Dombard et al. identified the Crenarchaeota as the most abundant archaeal group, whereas Thaumarchaeota were the most abundant group in the acidic Mutnovsky spring sample. Thaumarchaeota are the dominant archaeal group also in hot springs on the Tibetan Plateau (Huang et al. 2011). By contrast, the analysis of the

Uzon sample revealed a more diverse prokaryotic community than in the Mutnovsky sample, being dominated by uncultured members of the MCG and *Enterobacteriaceae*. Despite the geographical separation, the Obsidian Pool and the Uzon Caldera hot spring share a very similar community structure, as almost the same dominant archaeal and bacterial groups were identified, together with the rare phylum of Korarchaeota (Meyer-Dombard et al. 2005). These results confirm also the presumption proposed for other sites that similar extreme environmental conditions result in similar microbial communities (Simon et al. 2009).

5.1.3 Furnas Valley, Azores

Azores is a group of islands of volcanic origin in the Atlantic Ocean, with the main hydrothermal area being the Furnas Valley on the Island of São Miguel. The largest spring is at the highest elevation and is alkaline, whereas some of the lower springs are smaller and more acidic (Brock and Brock 1967). This is in opposition to the displacement in YNP and in Iceland where the higher springs are small and acidic and the lower ones are large and alkaline (Allen and Day 1935; Barth 1950). Sahm and co-workers have analyzed nine sites in the Valley of Furnas, (AI-AIV near Caldeira Do Esgucho, BI-BIII near Caldeirão and CI and CII near Caldeira Asmodeu) with a wide variety of physico-chemical characteristics with a range of temperature between 51 and 92 °C and pH values between 2.5 and 8.0 (Sahm et al. 2013) (Table 5.1). The study was performed through a mixed approach including the analysis of 16S rRNA, both by PCR amplification and metagenomic, fluorescence in situ hybridization (FISH), and denaturing gradient gel electrophoresis (DGGE) to estimate the prokaryotic diversity. The DGGE-profile on all the sites indicates that the pH showed the prominent effect on the microbial complexity in the different samples. Indeed, the lower diversity was observed in combination of extremes of acidic pH and temperature (pH 2.5–3.0 and 84 °C), while the highest microbial diversity was detected with temperatures between 55 and 85 °C and pH values between 7.0 and 8.0. The DGGE-profiles of archaeal 16S rRNA revealed an overall lower number of bands showing a quite identical pattern in the closely spaced sites with higher temperatures AIII and AIV (85 °C pH 8.0 and 92 °C pH 8.0, respectively). The samples AI (51 °C, pH 3.0) and AIV are connected with the outflow of AIV running into AI and show a wide diversity in temperature and pH. The samples were analyzed by sequencing of the 16S rRNA, by using the amplification of the regions V2/V3 with specific primers for Archaea and Bacteria, and by FISH by using domain-specific probes to quantify the relative abundances of Bacteria and Archaea. Sahm and co-workers reported that, by FISH, AI site was dominated by Bacteria (68% of total cells) and the Archaea could not be detected at all. By contrast, the site AIV showed that the relative contribution of the specific domains was almost 35% Archaea and 40% Bacteria (Sahm et al. 2013). The partial amplification of 16S rRNA genes using specific primers for archaeal and bacterial V2/V3 regions produced 93,576 clean sequences (74% of the total raw sequences)

with an average length of 390 bases. In addition, the sample AIV was successively analyzed by sequencing the overall 16S rRNA from the metagenomic DNA producing 725 partial 16S RNA genes sequences that were analyzed against SILVA database (Pruesse et al. 2007). The overall results indicate that the acidic spring AI was dominated by Proteobacteria (80%), prevalently of the acidophilic genera such as the heterotroph *Acidicaldus* (38%) and the chemolithoautotroph *Acidithiobacillus* (43%), and by the phylum of the Firmicutes (10%) related to *Anoxybacillus*. Instead, bacteria belonging to the phyla of Thermotogae, Firmicutes and Dictyoglomi, with the genera of the Fervidobacterium, Caldicellulosiruptor and Dictyoglomus, respectively, dominated the site AIV. These genera constituted up to 61% and 88%, regarding the metagenomic pyrosequencing and the amplified V2/V3 16s rRNA, respectively, of the bacterial community, indicating a high abundance of heterotrophic microorganisms. The sequencing of the metagenomic 16S rRNA of this sample also revealed high abundance of two genera from the phylum Aquificae, with the chemolithoautotrophic *Sulfurihydrogenibium* being the dominant genus (22%) (Sahm et al. 2013).

The archaeal community of the two sites showed substantial differences. The Archaea present in AI were only Euryarchaeota, mainly belonging to the genus Thermoplasma (89%) while the archaeal population of the site AIV was almost exclusively composed by Crenarchaeota, belonging to the family of Desulfurococcaceae (75%), with the genera *Sulfophobococcus* (55%) and *Desulfurococcus* (19%), and the family of Thermoproteaceae with the genus *Pyrobaculum* (25%).

A more detailed analysis of the 16S rRNA genes indicates that in AIV the partial sequences are more than 99% identical to those from cultivated organisms. The authors reported that for the genera of *Fervidobacterium*, *Dictyoglomus* and *Caldicellulosiruptor* more than 85% of the sequences showed an identity >99% to *F. islandicum*, *D. thermophilum*, and to *C. lactoaceticus* species, respectively, while only few sequences (11%) were 97% identical to cultivated or related to uncultivated species. A different situation was observed for AI where 94% of the *Acidicaldus*-specific sequences were related to uncultivated organisms and 99% of the *Acidithiobacillus*-sequences were 99% identical to *Acidithiobacillus caldus* (Sahm et al. 2013).

This global result is in contrast to the majority of other environmental studies, in which the largest proportion of sequences cannot be assigned to cultivated species. Moreover, the dominant genera *Caldicellulosiruptor*, *Dictyoglomus*, and *Fervidobacterium* in AIV site, have also been detected in in situ enrichment cultures in hot springs from Uzon caldera in Kamchatka (Kublanov et al. 2009). The first two genera are able to grow on polysaccharides (cellulose and chitin) while *Fervidobacterium* grows on proteinaceous substrates, suggesting that the Furnas hot springs could be a reliable source of new polymer-degrading enzymes and organisms.

16S rRNA analyses indicated the dominance of heterotrophic bacterial genera in both springs in contrast to many other studies where Aquificales have been repeatedly found to dominate. To explain this discrepancy, the authors suggest that chemolithotrophic physiology probably based on the oxidation of H_2 or reduced sulfur compounds is the major metabolic pathway in both samples and that,

depending on the chemical characteristics of the spring, different subgroups of Aquificales could be dominating. In particular, it was reported that high-sulfide and sometimes iron-rich habitats were dominated by Aquificales branches J and S which today are attributed to *Sulfurihydrogenibium* (Hugenholtz et al. 1998; Yamamoto et al. 1998; Reysenbach et al. 2000). According to Sahm and colleagues, the high abundance of *Sulfurihydrogenibium* (9 and 22%, by FISH and metagenomic respectively) from site AIV fitted into the picture. The low relative abundance of chemolithoautotrophic organisms at site AIV might be related to the high DOC concentration of 284 mg/L, while data from other hot springs where a clear dominance of Aquificales was observed indicate DOC contents between 0.41 and 10 mg/L (Yamamoto et al. 1998; Hetzer et al. 2007; Hall et al. 2008). Thus, a 20–400-fold higher concentration of DOC in the Furnas spring could be a reason for the abundance of heterotrophic bacteria. About the Archaea in the site AIV, the metagenomic rRNA data suggest that the heterotrophic genera *Sulfophobococcus* spp. and *Desulfurococcus* spp. represent approximately 74% of the sequences while the remaining 26% could be related to chemolithotrophic genera *Pyrobaculum* (25%), *Stetteria* (0.5%) and *Staphylothermus* (0.5%) (Sahm et al. 2013).

The study of Sahm and collaborators represents the state of the art on the characterization of the microbial communities in the Furnas springs which, according the authors, reflect a natural enrichment in this area of heterotrophic and polymer degrading genera that could be promising for the search of new thermostable biocatalysts for biotechnological applications.

5.1.4 Malaysian Sungai Klah

The Sungai Klah (SK) hot spring, the second hottest geothermal spring in Malaysia, is a shallow, 150 m long, fast-flowing stream, with temperatures varying from 50 to 110 °C and a pH range of 7.0–9.0. Hidden within a wooded area, the SK hot spring is continually fed by plant litter, resulting in a relatively high degree of total organic carbon (TOC). In 2015, Chan and coworkers studied the middle of SK stream (75–85 °C, pH 8.0) by performing a metagenomic analysis of a mixture of water and sediment sample (Chan et al. 2015) (Table 5.1). The sample extracted from this site was analyzed by 16S rRNA sequencing and shotgun analyses. For the metagenome sequencing the Illumina HiSeq 2500 sequencer and a dual-indexed 151 (Paired-End sequencing) strategy was used. Paired-end sequencing reads were filtered with the Trimmomatic 0.30 trimming tool (Bolger et al. 2014) for a minimum terminal base quality score of 20 and a length >30 bp. *De novo* assembly was performed using the IDBA-UD assembler and all contigs <300 bp were discarded. This strategy allowed obtaining a total of 278,434 contigs with coverage exceeding 10X. The authors found that 88.44% of the predicted ORFs belonged to Bacteria, and 10.14% and 0.67% were from Archaea and Eukaryota, respectively. A small fraction of the total contigs belonged to viruses and unclassified sequences. The metagenome shotgun sequencing approach allowed the identification of 83 phyla, the top 6 of which are

Firmicutes, Proteobacteria, Chloroflexi, Bacteroidetes, Euryarchaeota and Crenarchaeota. This distribution of the major phyla was similar between both the 16S rRNA and shotgun metagenome approaches. A total of 1,203,458 full-length protein-coding genes identified within the shotgun metagenome dataset were analyzed and, among these, 817,831 ORFs were annotated and classified.

Interestingly, Chan and coworkers used the sequence affiliations to understand the relationship between the geochemical parameters and the population diversity within this hot spring. They showed that the SK hot spring community uses diverse means for growth, as suggested by the analysis of carbon metabolism, since a fraction of the community exhibits a complete metabolic pathway, whereas the others may benefit from syntrophic relationships. In addition, microorganisms in the SK community contain more than one carbon fixation pathway thus allowing the use of different inorganic carbon sources. The community appears to survive using mutualistic or commensalistic symbiotic relationships to thrive under multiple environmental stresses. The authors suggested that the uniqueness of the diversified pathways observed is likely a result of the physical characteristics of the hot spring and of additional factors, such as dissolved gases, minerals, and trace elements. The SK hot spring is richer in aluminum, iron, sulfate, and sulfur in comparison to other Malaysian hot springs. The authors compared SK hot spring to 60 other Malaysian sites, and reported that it is unique due to the natural environment of the site. They identified four key factors; (i) the stream contains multiple spring pools with temperatures exceeding >100 °C; (ii) temperature along the streams fluctuate from 50 to 110 °C; (iii) pH along the streams is not uniform and ranges between 7.0 and 9.0, and (iv) the SK hot spring is fed with plant litters that enhance its carbon contents. The analysis of the microbial diversity present in another hot spring named Little Hot Creek (LHC) (78.7–82.5 °C and pH 6.75–6.97), determined by 16S rRNA analysis, revealed that the dominant phyla are Aquificae, Thermodesulfobacteria, Deinococcus–Thermus, Thermotogae, Chloroflexi, and Dictyoglomi (Vick et al. 2010). This is in contrast with SK, where approximately 56% of the phyla were composed of Firmicutes and Proteobacteria. As suggested by the authors, the biodiversity of the SK hot spring is due to combinations of the three aforementioned factors (i–iii) and to the plant litter enriching the SK microbiome diversity of thermophiles by providing additional carbon sources.

5.1.5 Tengchong, China

One of the most active geothermal areas in the world is Tengchong in China, which is located on the northeastern edge of Tibet–Yunnan geothermal zone between the Indian and Eurasian plates. The Rehai (“Hot Sea”) and Ruidian geothermal fields in Tengchong are two regions of intense hydrothermal activity with numerous springs and pools (Table 5.1). Physicochemical conditions span a wide range of temperature (58–97 °C) and pH (1.8–9.3) (Hou et al. 2013). Rehai harbors various types of hot springs: small source, high discharge springs such as Gumingquan and

Jiemeiquan; small, shallow acidic mud pools, such as those in Diretiyanqu, that formed a decreasing temperature gradient; shallow acidic pool Zhenzhuquan; and shallow spring with multiple geothermal sources such as Shuirebaozha. Large pools with neutral pH such as Gongxiaoshe and Jinze are located in the Ruidian geothermal field (Wang et al. 2014). Based on physical characteristics, the hot springs can be divided in 4 groups: (i) high temperature and neutral-alkaline pH (in both Rehai and Ruidian regions), (ii) moderate temperature and neutral pH (Ruidian), (iii) low temperature and low pH (Rehai), (iv) high temperature and low pH (Rehai). In 2013, Hou and co-workers published the first comprehensive census of the microbial community in 16 different hot springs of Tengchong. Previously, few metagenomic studies focused only on Crenarchaeota (Song et al. 2010) or ammonia oxidizing archaea (AOA) (Jiang et al. 2010) have been reported. Hou's work focused on the relationship between thermophilic microbial communities and geochemical conditions. The microbial community was analyzed through PCR amplification of the bacterial and archaeal V4 V8 variable regions and sequencing of the 16S rRNA genes. The sequences were aligned with the PyNAST method (Caporaso et al. 2010a) and operational taxonomic units (OTUs) were identified with Chimera Slayer (Haas et al. 2011). More than 90% of total sequences from Rehai springs were composed of Archaea, mainly Crenarchaeota. Desulfurococcales and Thermoproteales (mainly *Pyrobaculum*) were the dominant orders present in the springs with neutral-alkaline pH (pH 6.7–9.4) and high concentrations of silica, Na^+, K^+ and Cl^+. Instead Sulfolobales, predominantly the genus *Sulfolobus*, were dominant in high temperature, acidic, and sulfur-rich springs (85.1–89.1 °C and pH 2.5–4.8). Within acidic sites, temperature exerted a strong control on community composition. With decreased temperature, a Sulfolobus-dominated community was replaced by the bacterial taxa *Hydrogenobaculum*, with Aquificae as the most abundant bacterial phylum. Some bacterial and archaeal groups, such as *Hydrogenobaculum* and *Sulfolobus*, were found only in Rehai, whereas *Thermaceae* and *Rhodothermaceae* were fairly abundant only in Ruidian. Putative ammonia-oxidizing *Thaumarchaeota* were the dominant archaea in Ruidian.

The correlation between microbial diversity and environmental geochemistry was measured by Chao1 (predicted number of OTU), Shannon and equitability indices based on 16S rRNA gene sequence data. These diversity indices were tested for their correlation with the geochemical data using Mantel test. Higher microbial richness, equitability, and diversity in Ruidian than in Rehai were found. According to Hou et al., this might be due to the neutral pH, moderate temperatures, and high TOC contents as well as the different mineralogy (carbonates and silicates) of the Ruidian springs relative to those from Rehai (Hou et al. 2013).

More recently, the correlation between microbial diversity and geochemistry in Tengchong has been analyzed by considering the seasonal changes (Wang et al. 2014), since the hot springs in Tengchong are located in a subtropical area with heavy temporal monsoon rainfall (Briggs et al. 2014). Wang and co-workers compared their samples collected in the rainy season (June and August) with the analysis of Hou and colleagues obtained by samples collected in the dry season (January). They found that the seasonal effects on the microbial diversity are more

pronounced in sediment relative to water sample. In acidic springs the water communities between January and June were highly similar to each other and both were predominated by *Sulfolobus*. Instead, in August, *Hydrogenobacter* became the most abundant taxon followed by *Sulfolobus*. In the sediments of two acidic springs, named Zhenzhuquan (89.1 °C, pH 4.79) and Diretiyan-1 (85.1 °C, pH 2.58), from January to August the dominant taxa *Sulfolobus* was replaced by *Desulfococcus* and *Ignispaera*, respectively. In the neutral-alkaline spring, the water community remained constant in both dry and rainy seasons, except for the *Shuirebaozha* spring (79.8 °C, pH 8.28) where, among the most abundant taxa, *Fervidobacterium* changed in *Hydrogenobacter* from June to August, while *Persephonella* remained constant over time. The two alkaline springs, characterized by fast-flowing and high discharges, harbored *Hydrogenobacter* in January and June but the community structures showed a notably change in August, where *Persephonella* and the candidate phylum OP1 became the dominant members. Both studies revealed that Ruidian sediments contained more diverse microbial lineages than Rehai sediments thanks to the neutral pH and moderate temperatures. Specifically, the neutral spring contained similar microbial lineages in January and June, but in August a single dominant lineage of *Thermus* emerged.

The exact reasons of the seasonal changes in microbial community structure were difficult to establish. Wang and co-workers suggested that pH was a primary factor influencing the microbial community shifts, followed by temperature and DOC. For example, in Zhenzhuquan, where the pH ranged from 4.8 in January to 6.1 in August, the change of dominant microbial lineages from *Sulfolobus* to microaerophilic *Hydrogenobacter* (in the water) and anaerobic *Desulfococcus* (in the sediments) was observed. Indeed, the pH 6.1 exceeded the growth pH range for *Sulfolobus* (0.9–5.8) (Brock et al. 1972) but fits well with the pH range of *Hydrogenobacter* (near neutral pH) (Takacs-Vesbach et al. 2013) and *Desulfococcus* (pH 6.0–6.5) (Faith 1992). Instead, in the other acidic spring Diretiyanqu-1 the shift of dominant taxa from *Sulfolobus* to *Ignisphaera* was not driven by the increased pH but by the high accumulations of NH_4^+, K^+, and Na^+ ions that could inhibit the growth of *Sulfolobus* species by affecting their RNA polymerase activity (Park and Lee 1999).

Microbial community composition and its correlation with geochemistry in Tengchong could be better understood by a comparison to other geothermal systems in the world. In terms of mechanisms and genesis, the Tengchong hot springs are very similar to those in YNP in that they are both volcanically driven. As a result, many springs in YNP and Tengchong, in particular in Rehai, harbored similar microbial communities at the phylum and family/genus levels such as the bacterial and archaeal phyla Aquificae and Crenarchaeota, respectively. In more detail, four genera of *Aquificales* were present in both Tengchong and YNP springs (Hou et al. 2013) but their relative abundance was highly different. For example, in neutral alkaline springs (>78 °C) *Hydrogenobacter* dominated on *Thermocrinis*, although, the former was more abundant at lower temperatures (60–80 °C) than *Thermocrinis* (75–92 °C) (Eder and Huber 2002). Instead, *Sulfurihydrogenibium* is more scarcely present in Tengchong than YNP springs (Inskeep et al. 2005;

Yang et al. 2011). Hou and coworkers suggested that this may be related to maximum growth temperature of 78 °C of *Sulfurihydrogenibium*, which was below the lowest temperature in the neutral and alkaline samples (78–90 °C), except Ruidian springs (74–82 °C). In the latter springs, however, the relatively high dissolved O_2 content may be incompatible with the microaerophilic nature of *Sulfurihydrogenibium* (Nakagawa et al. 2005). *Deinococcus-Thermus*, the most abundant species in Ruidian spring (73.8 °C, pH 7.29), has a geochemical distribution similar to YNP and the crenarchaeal class Thermoprotei is largely abundant in Rehai and in many sites in YNP (Meyer-Dombard et al. 2005).

In conclusion, the two geothermal fields Rehai and Ruidian were highly diverse in their environmental conditions, in terms of temperature range (55–94 °C) and pH (2.5–9.4). The bacterial phylum Aquificae and the archaeal phylum Crenarchaeota are dominant in all Rehai samples and in the water samples of Ruidian but their specific compositions are highly dependent on geo-physical characteristics of the springs and seasonal physicochemical changes (Hou et al. 2013; Wang et al. 2014). Therefore, these studies suggest that the temperature, the pH and other geochemical conditions play a key role in shaping the microbial community structure in Tengchong hot springs.

5.1.6 *Taupo Volcanic Zone, New Zealand*

The Taupo Volcanic Zone (TVZ) consists of a complex group of high temperature geothermal systems in the central North Island of New Zealand. One of the major geothermal fields in the TVZ is Waiotapu, which is characterized by a large number of springs (Table 5.1) with elevated arsenic concentrations (Mountain et al. 2003). The largest feature at Waiotapu is Champagne Pool (CP), ~65 m in diameter with an estimated volume of ~50,000 m^3 (Hedenquist and Henley 1985), and an arsenic concentration between 2.9 and 4.2 mg L^{-1}. The inner rim of CP is characterized by subaqueous orange amorphous As-S precipitate (Jones et al. 2001). The narrow outflow channel (~40 cm wide and 5 cm deep), in a sub-aerial sinter dam, drains the spring water out across a shallow siliceous sinter terrace. Convection in CP stabilizes water temperature at 75 °C, while in the surrounding silica terrace ("Artist's Palette"), the temperature decreases to 45 °C. Water-rock interactions beneath the pool lead to silica dissolution and sulfide oxidation (Ellis and Mahon 1967) and provide sources of acidity to CP waters. In 2014, Hug and co-workers studied the microbial contributions to coupled arsenic and sulfur cycling at Champagne Pool, with implications for understanding the evolution of microbial arsenic resistance in sulfidic geothermal systems. In this study, the hot spring was divided in four sampling sites on the basis of distinctive physical and chemical characteristics. These sites were located along a natural hydrologic gradient from the inner pool (CPp) through the inner rim (CPr) and outflow channel (CPc), with pH ranging between 5.5 and 5.8, and on to an outer silica terrace (AP), pH 6.9. Total dissolved arsenic concentrations of 3.0, 2.9, 3.6, and 4.2 mg L^{-1} were measured at

sites CPp, CPr, CPc and AP, respectively. All sampled sites contained total dissolved sulfur concentrations between 91 and 105 mg L^{-1} (Hug et al. 2014). High temperatures and high concentrations of dissolved toxic metal(loid)s determine a strong selective pressure on extant microbial communities. Indeed sulfide, elemental sulfur, thiosulfate, and sulfate are common electron donors or acceptors for microorganisms under hydrothermal conditions (Macur et al. 2013), and sulfide ions are highly reactive with arsenic. Indeed, Stauder and co-workers suggested the transformation of arsenite into thioarsenates via elemental sulfur indicating that, microbially-mediated sulfur cycling could exert a profound, although indirect, influence on arsenic speciation, by thioarsenate species control (Stauder et al. 2005). In comparison to arsenite and arsenate, thioarsenates are considered to be less toxic for microorganisms, as the sulfurarsenic bond leaves no free electron pair to bind with sulfhydryl-groups in amino acids. Despite in the work by Planer-Friedrich et al. (2008) thioarsenate species were identified as potentially toxic to microorganisms over longer exposure times, it has been demonstrated that many microbes employ a range of strategies to detoxify arsenic (Planer-Friedrich et al. 2008). The most ubiquitous arsenic resistance mechanism is the expression of the *ars* operon encoding for proteins that identify and transport arsenic (Páez-Espino et al. 2009).

To determine potential microbial contributions to arsenic speciation in CP and to characterize the microbial diversity, Hug and co-workers extracted total genomic DNA from sediments and the samples were sequenced using Illumina Miseq. Sequence analysis was performed using the rapid annotation subsystems technology for metagenomes (MG-RAST) bioinformatics package. The numbers of clean reads obtained by the sequencing were about 2 million for CPp, CPr and CPc and about 4 million for the AP sample. For taxonomic analysis, 16S rRNA gene sequence data were compared to all accessory databases. These analyses showed that the lowest species abundance was detected at CPc, with species abundances of CPp and CPr closer to CPp than to AP. At CPp, 12% of the sequences belonged to Archaea, consisting almost exclusively of *Thermofilum*, *Sulfolobus* and *Pyrobaculum* (together 8%). Instead, 16S rRNA gene sequences resulted ~21–28% of Archaea at CPr and CPc, respectively, mostly assigned to genera *Sulfolobus, Thermofilum, Pyrobaculum, Desulfurococcus, Thermococcus,* and *Staphylothermus*. In AP only 2% of the sequences belonged to Archaea, with no dominant genus present. Hug reported that, across all sites, most abundant sequences belonged to Bacteria closely related to the genus *Sulfurihydrogenibium,* in particular 19% at CPp and CPr, 13% CPc and 10% AP. Additionally, bacterial genera *Anoxybacillus* and *Persephonella* comprised 38 and 3% of the total sequences at CPp, respectively; whereas at AP, alongside *Sulfurihydrogenibium, Thiomonas* and *Thermus* were the most abundant bacterial genera in the community with 9% and 4% of total sequences.

Hug and colleagues reported that microbial community, including a large group of sulfur-cycling microorganisms, increased its richness with decreasing temperatures and increasing pH. They suggested that this could be an evidence of the indirect biological mediation of arsenic speciation via microbial sulfur cycling (Ullrich et al. 2013; Hug et al. 2014). At CPp, the main proportion of sulfur

metabolizing genes belonged to sulfur oxidation genes, whereas at CPr and CPc belonged to sulfur reduction genes. Instead at AP the proportion of sulfur metabolizing genes changed again to sulfur oxidation genes. According to the authors, the combination of sulfide dehydrogenase and sulfur oxygenase-reductase encoding genes, detected as major sulfur oxidation genes at CPp, suggests a two-step sulfide oxidation process to sulfite and thiosulfate, also producing sulfide. Thiosulfate could be further oxidized via oxygen at the surface of the pool. The annotation of sulfur oxidation genes detected at CPp revealed a close relationship to the genus *Sulfolobus*. This sulfur-oxidizing genus (Brock et al. 1972) enhances the potential for production of thiosulfate and sulfate. Other 16S rRNA gene sequences detected in CPp were closely related to members of the order *Aquificales*, primary producers in high temperature ecosystems (Eder and Huber 2002) and capable of oxidizing H_2 or reduced sulfur species. The presence of sulfur reduction genes at CPp, belonging to the *dsr* and *asr* gene complexes, is consistent with the thiosulfate or elemental sulfur-reducing genus *Pyrobaculum* (Stetter et al. 1990). The resulting biogenic sulfide produced, would then be available to transform arsenite to monothioarsenate and yielding H_2. At CPr the increase in sulfur reduction genes is consistent with the detection of close relatives from the genera, which have the potential to reduce thiosulfate or elemental sulfur to sulfide. Since the sulfide concentration at Cpr did not increase significantly, Hug and collaborators assumed that biogenic sulfide was probably rapidly reoxidized via sulfur oxidation by *Sulfolobus* (Hug et al. 2014). In addition, the authors observed that alongside the indirect impacts on arsenic transformation from microbial sulfur cycling, the metagenomic data for all sites revealed the presence of arsenic resistance genes. The dominance of the *ars* operon supports the high degree of utility and conservation of this arsenic resistance mechanism (Hug et al. 2014).

In conclusion, phylogenetic analysis of 16S rRNA genes from metagenomic sequencing revealed the dominance of *Sulfurihydrogenibium* at all sites and an increased archaeal population at the rim and outflow channel. Several phylotypes were found closely related to known sulfur- and sulfide-oxidizers, as well as sulfur- and sulfate-reducers. Bioinformatic analysis revealed genes underpinning sulfur redox transformations, consistent with sulfur speciation data, and illustrating a microbial role in sulfur-dependent transformation of arsenite to thioarsenate. Metagenomic analysis also revealed genes encoding for arsenate reductase at all sites, reflecting the ubiquity of thioarsenate and a need for microbial arsenate resistance despite anoxic conditions.

5.1.7 Phlegraean Fields, Italy

The Phlegraean Fields is a large volcanic area situated to the west of Naples, Italy. The area of the caldera comprises 24 craters and volcanic features, lying mostly underwater and showing hydrothermal activity at Lucrino, Agnano and in the town of Pozzuoli.

Solfatara volcano is located in the central part of the repeatedly collapsed Phlegraean Fields caldera, and is one of the youngest volcanoes formed within this active volcanic field (Rosi and Santacroce 1984; Orsi et al. 1996; Isaia et al. 2009). It is a tuff cone located at 100 m above sea level, about 2 km off the town of Pozzuoli. The central part of the crater is occupied by the "Fangaia mud pool", at which the water table emerges and a continuous rising of hydrothermal fluids generates diffuse bubbling (Table 5.1) (Petrosino et al. 2012).

Pisciarelli spring is an area with intense hydrothermal activity and currently exhibits one of the most impressive degassing manifestations. A direct relationship between the increase of hydrothermal activity in the Pisciarelli solfataric area and ground uplift in the caldera has been observed. For this reason, its activity is considered a direct indicator of the volcanic dynamics (Troiano et al. 2014). Pisciarelli spring is only about 800 m^2 in size, but contains more than 20 physically and chemically different springs and mud holes (Table 5.1). A typical feature of continental solfataric fields is the existence of two dominating zones in the soil. The upper oxidized zone is an orange-colored Fe-oxid mat. Generally, the thickness depends on the volcanic activity and exhalation of reducing volcanic gases such as H_2S and H_2, and is between a few centimeters and about 50 cm. Under the oxidized layer, there is a black-colored anoxic zone rich in ferrous sulfide. The surface between these zones, with a width of a few millimeters, is slightly yellow-colored and characterized by the presence of elemental sulfur. This element is formed by chemical oxidation of H_2S coming from below by molecular oxygen penetrating from the surface into the soil. In this high-temperature environment, beyond sulfide, arsenic is one of the most prominent heavy metals (Huber et al. 2000). This suggests the existence of hyperthermophiles, in arsenic rich environment, able to use arsenic compounds in their metabolism, as Hug and co-workers found in Champagne Pool (New Zealand) (Hug et al. 2014). Previous phylogenetic analyses of a highly acidic sample from Pisciarelli (original temperature 95–97 °C) showed archaeal 16S rRNA sequences belonging to the *Sulfolobales* and *Thermoplasmatales*, which are known to grow optimally between pH 1.0 and 3.0 (Huber et al. 2000). In particular, *Sulfolobus solfataricus* strain P2 was isolated for the first time from Pisciarelli (Zillig et al. 1980).

In 2015 Menzel and colleagues analyzed Solfatara volcano (It6) and Pisciarelli hot spring (It3) by metagenomic approach aiming to define the biodiversity, genome contents and inferred functions of bacterial and archaeal communities (Menzel et al. 2015). It6 sample (76 °C, pH 3.0, water/sediment) was sequenced by Illumina Hiseq and analyzed for estimating species abundances by MEGAN (Huson and Weber 2013). From this sample 78.6% of the mapped reads were assigned to Bacteria and 17.6% to Archaea. The most abundant phyla were Proteobacteria (72%) and Thermoprotei (15%). The former was mostly comprised of the genus *Acidithiobacillus* (64%) and a smaller number of Firmicutes (4%). Archaea were composed by Crenarchaeota (15%), with *Acidianus hospitalis* being the most abundant species (4%), and Euryarchaeota (3%), with 2% of the reads assigned to *Ferroplasma acidarmanus*. By the assembly of the reads, Menzel et al. reported 8 distinct 16S rRNA archaeal sequences. Among these, two sequences were assigned

to *Acidithiobacillus*, three to *Sulfolobus*, two to *Sulfobacillus* species. One predicted full-length 16S sequence showed 99.8% identity to *Ferroplasma acidarmanus*. According to the authors, the metabolism of these genera reflected the organic and inorganic content of the pool; for example, the aerobic and autotrophic bacterium, *A. thiooxidans* is a sulfur oxidizer, acidophilic, chemoautotrophic whose carbon requirements are fulfilled by CO_2 from the atmosphere (Ko et al. 2013; Menzel et al. 2015). Instead *F. acidarmanus* is iron-oxidizing archaeon.

In addition, Menzel and co-workers analyzed Pisciarelli site (It3) (86 °C, pH 5.5, water/sediment). Total genomic DNA was extracted from sample and sequenced by Roche/454 Titanium FLX. To estimate the species abundance MEGAN was used to analyze the cleaned reads (about 680,000). It3 almost exclusively contains Archaea (96.6%), with 50% of clean reads assigned to *Acidianus hospitalis* and 32% to *Pyrobaculum* species. These data have been confirmed by 16S rRNA sequences analysis. Among the 9 identified sequences, 3 were >98% identical to *Acidianus* species, including one full length with >99% identity to *Acidianus hospitalis W1*. Moreover, 3 sequences showed highest identity to *Pyrobaculum* species (Menzel et al. 2015).

In conclusion, Menzel and colleagues suggested that the community structure was largely determined by a combination of environmental parameters, rather than geographical distance. Indeed, It3 sample (86 °C, pH 5.5) was almost exclusively comprised of Crenarchaeota (30% of *Pyrobaculum* species), which also corresponded to other moderately acidic pools in YNP (79 °C, pH 1.8) (Inskeep et al. 2013a). In contrast, the sample It6 (76 °C, pH 3.0) was largely comprised of species belonged to the bacterial genus *Acidithiobacillus*, which is known to be an acidophilic mesophile. However, members of this genus have recently been observed in a hydrothermal spring (51 °C/pH 3) of the Azores (Sahm et al. 2013), in a highly acidic river metagenome (59 °C/pH 1) from Argentina (Sofía Urbieta et al. 2014) and in a thermoacidophilic hot pool (70 °C/pH 3.5–4) at the Mutnovsky volcano (Wemheuer et al. 2013).

5.1.8 Metagenomics of Hydrothermal Sites to Access to Novel Enzymes for Biotechnology

The sources of new enzymes were technically limited to a minor fraction of total microbial diversity, the culturable microorganisms, which have been estimated as representing less than 1% of the real diversity in most environments (Amann et al. 1995). This drawback is even more prominent with extremophilic microorganisms. Thus, metagenomics of extreme environments play a key role in the discovery of new enzymes with unique feature combinations crucial for industrial development. Indeed, thermostable enzymes from thermophilic microorganisms are important biocatalysts for industrial and biotechnological purposes, given that they can work at high temperatures in which mesophilic enzymes would be denatured. Two types of protein thermostability are of interest for industrial purposes: thermodynamic

stability (when an enzyme is used under denaturing conditions such as high temperature or presence of an organic solvent) and long-term stability (Sharma et al. 2012). Enzymes from thermophiles, often show both types of thermostability and are able to resist also at the combination of different denaturants (temperature and organic solvents, detergents and extreme pH values) (Haki and Rakshit 2003). Due to these unique properties, thermozymes are of tremendous importance for biotechnological applications and, therefore, screening for novel biocatalysts from extremophiles represents a valuable alternative to elaborative engineering procedures for the optimization of available enzymes from mesophiles (Cobucci-Ponzano et al. 2015).

Since the classical example of Taq DNA polymerase from *Thermus aquaticus,* purified and isolated from hot springs (Chien et al. 1976), which made the development of the PCR amplification technique possible, there are now many thermophilic enzymes being used for biotechnological and industrial purposes. Studies of the biodiversity in hot springs revealed the presence of complex communities containing novel microorganisms, which can be potential sources of novel enzymes with unique features of interest in industrial applications. Indeed, thermophiles and hyperthermophiles produce a variety of hydrolytic enzymes such as lipases, glycosidases, peptidases, which are of applicative interest. Thermophilic enzymes were primarily screened in a culture-based manner, but bioprospecting of extreme temperature metagenomes by either homology based DNA sequence data mining or functional screening of metagenomic DNA libraries is currently regularly providing new thermostable biocatalysts with potential in biotechnology applications. Heterotrophic (hyper)thermophilic prokaryotes are capable to utilize various polymeric substrates as carbon sources. A resourceful enzyme repertoire that is stable at specific extreme environmental conditions facilitates the efficient degradation of complex natural polymers including starch, lignocellulose, chitin as well as proteins and fats (Elleuche et al. 2015). In recent years biocatalysts search in metagenomic DNA libraries from hot springs was mainly focused on the discovery of esterases, lipases and glycosidases, instead only one DNA polymerase and one protease were reported. We here describe some recent examples of new thermostable enzymes with high potential in biotechnological applications identified from high temperature metagenomes (for a review see López-López et al. 2013; Lewin et al. 2013).

Viral metagenomes are an unexplored source of sequence diversity for the development of new enzymes. Moser and coworkers constructed a viral metagenomic library from Octopus hot spring in YNP and analyzed 21,198 Sanger sequence reads. BLASTx alignment to the Genbank protein sequence database identified hundreds of potential polymerase genes. Among these, they identified the DNA polymerase 3173 with both high thermostability and innate reverse transcriptase (RT) activity. An easier-to-use exonuclease-deficient derivative was incorporated into a PyroScript RT-PCR master mix and compared to one enzyme (Tth) and two-enzyme (MMLVRT/Taq) RT-PCR systems for quantitative analyses. Specificity and sensitivity of 3173 Pol-based RT-PCR were higher than Tth Pol and comparable to three common two-enzyme systems. The performance and simplified

set-up make this enzyme a potential alternative for research and molecular diagnostics (Moser et al. 2012).

Lipases and esterases are ubiquitous in nature and can be found in animals, plants and microorganisms, but most industrial lipases are of microbial origin. Nowadays, lipases and esterases represent a major portion with high growth potential in the World Industrial Enzymes Market. They have many applications in the food and paper industry, synthesis of biopolymers, biodiesel production, synthesis of fine chemicals, bioremediation and waste treatment. Most industrial processes in which lipases/esterases are used as biocatalysts are carried out at temperatures above 45 °C and in the presence of organic solvents. Lipases from extremophiles can provide special features that make them more suitable for specific applications where a lipolytic biocatalyst is required (López-López et al. 2014). Several thermostable esterases have been isolated in recent years by functional screening of metagenomic libraries from hot spring. EstE1 and Est1 were identified from a functional screening of metagenomic library of samplings in the Sileri region of Indonesia (80–95 °C, pH 4.0–6.0) (Rhee et al. 2005) and of Jae Sawn hot spring in Thailand (70 °C, pH 7.0) (Tirawongsaroj et al. 2008), respectively. These enzymes display typical thermophilic profiles: extremely stable at 80 and 70 °C in the absence of any stabilizer, with high optimal temperatures of 95 and 70 °C, respectively. The activity at lower temperatures is remarkably high in EstE1: 20% and 30% of its optimal activity is retained at 30 and 40 °C, respectively. In addition, from the functional screening of the same Jae Sawn hot spring metagenome, Tirawongsaroj and coworkers identified a novel patatin-like phospholipase (PLP) containing four conserved domains, similar to other patatin-like proteins with lipid acyl hydrolase activity, and exhibiting high *V*max toward *p*-nitrophenyl butyrate. PLP and Est1 enzymes had activity toward both short-chain (C4 and C5) and long chain (C14 and C16) fatty acid esters. Therefore, PLP and Est1 are novel lipolytic enzymes from unculturable microbes, different from other known patatin-like phospholipases and esterases, which usually show no activity for substrates longer than C10 (Tirawongsaroj et al. 2008). More recently, three novel genes encoding lipolytic enzymes (*plpBW1*, *estBW1*, and *estBW2*), including a new patatin-like protein, have been identified by the functional screening of the metagenomic library prepared by Wemheuer and coworkers from the Uzon caldera in Kamchatka peninsula (see above). The closest relatives of all identified protein sequences originated from known thermophiles. They were similar to uncharacterized putative gene products derived from *Sulfurihydrogenibium azorense* (PlpBW1 and EstBW2), and *Thermobaculum terrenum* (EstBW1). The characterized lipolytic enzymes (PlpBW1, EstBW1, and EstBW2) showed features similar to those of other metagenome derived esterases, which were identified in thermophilic sites (Wemheuer et al. 2013).

The functional screening of the metagenomic library from the Uzon caldera allowed the identification of first metagenome-derived peptidase from a thermophilic environment (Wemheuer et al. 2013). The authors identified a gene sequence (*pepBW1*) that is almost identical to that of a putative gene encoding a serine peptidase of *Desulfurococcus kamchatkiensis*, belonging to the

Crenarchaeota, whose 16S rRNA gene sequence was found in the 16S analysis of the Uzon sample. The serine peptidase PepBW1 was affiliated to the subtilisin family (family S8). The recombinant *E. coli* strain containing PepBW1 was tested towards different proteins and showed proteolytic activity with skim milk and elastin-Congo red but not with azoalbumin or azocasein; however, no more enzymatic details are reported (Wemheuer et al. 2013).

Production of biofuels from the renewable lignocellulosic biomass is gradually considered as a promising way to replacement of fossil fuels. However, its bioconversion has been limited by the saccharification step because the main components of the lignocellulosic biomass (cellulose, hemicellulose and lignin) are tightly held together. Enzymatic hydrolysis is the most common process to degrade the cellulose and hemicellulose into fermentable sugars such as glucose and xylose. Many extreme thermophiles are able to utilize a variety of carbohydrates pertinent to the conversion of lignocellulosic biomass to biofuels. Identification and characterization of the glycoside hydrolases from these extremely thermophilic microorganisms is likely to generate new opportunities for the use of renewable resources as biofuels (Blumer-Schuette et al. 2008). Recently, a family 1 β-glucosidase (Bgl1) of archaeal origin was isolated by functional screening of a metagenome from the hot spring Caldeirão on the island São Miguel (Azores, Portugal) (Schröder et al. 2014). The samples collected at Furnas Valley, with temperatures ranging from 60 to 70 °C, pH 6.0–7.0, included water, mud and sediment. The putative protein Bgl1 exhibited 50–53% identity to putative glycoside hydrolases from species belonging to the class *Thermoprotei* of the phylum Crenarchaeota. The recombinant enzyme showed a broad substrate spectrum with activity toward cellobiose, cellotriose and lactose with a Ki value for glucose of 150 mM. Compared to most enzymes, extremely high specific activity with 3195 U/mg was observed at 90 °C and pH 6.5. Bgl1 was completely stable at pH 4.5–9.5 for 48 h at 4 °C and more than 40% of activity was measured at 105 °C. These distinctive characteristics distinguish Bgl1 from other enzymes described so far and make this enzyme suitable for application in numerous biotechnological applications that run at high temperatures (Schröder et al. 2014). To identify novel enzymes to be used in second generation biofuel technology, a very interesting approach based on the enrichment of microbial consortia on a selected biomass has been reported by Graham and collaborators (Graham et al. 2011). The authors assumed that given that no characterized hyperthermophilic archaeal species contain a minimum set of exo-, and endo-hemicellulases required to grow on lignocellulose above 90 °C, isolating a single cellulolytic species could be problematic. Hence, they set out to reconstitute a consortium of hyperthermophiles that could deconstruct lignocellulosic biomass at 90 °C. The sample was collected at Great Boiling Springs, a circumneutral geothermal pool at 94 °C near Gerlach, Nevada. The sediment was inoculated into minimal salts medium with the pulverized *Miscanthus gigas* as the primary carbon and energy source; then, after 3 weeks at 90 °C, a secondary enrichment with microcrystalline cellulose and a tertiary step with strips of Whatman No. 3 filter paper as a sole carbon source were set up. After

this enrichment strategy, since repeated efforts to separate the individual species were unsuccessful, they analyzed the consortium as a whole for potential cellulases of interest. The analysis of the metagenome of this consortium allowed the identification of a multidomain cellulase, the most thermotolerant endoglucanase reported to date, with a unique domain architecture. This approach, a compromise between environmental metagenomics and classical microbial isolation, resulted in the selection of a limited archaeal enrichment capable of growing on crystalline cellulose at 90 °C. The authors found that the consortium enriched on Avicel consisted of three strains but the closest characterized organism to the dominant member is related to *I. aggregans* DSM17230, representing a divergent new species of this genus. Since single isolates from this enrichment were not possible to obtain, the authors suggested that the diverse enzymes needed for lignocellulose utilization (cellulases, cellulose binding domains, xylanases, and cellobiohydrolases) do not allow for the survival of a single isolated hyperthermophilic Archaeon (Graham et al. 2011).

The studies surveyed in this paragraph prove that sequence-based and functional metagenomics are powerful strategies to discovering new hyperthermophilic enzymes with unique combinations of biochemical features and potential use in industrial applications. In the sequence-based metagenomic approach, new enzymes are discovered by exploring metagenomic data for enzymes homologous to known activities. Another common strategy is a PCR-based method with degenerate primers designed according to the conserved regions of already-known classes of enzymes. A disadvantage is that both approaches tend to detect only enzymes related to previously reported families, and might overlook those with completely new sequences. However, it is well documented that single amino acid differences may have drastic influences on enzyme properties and substrate recognition. Alternatively, metagenomic libraries can be subjected to functional screening to detect clones that exhibit the enzymatic activities of interest. The advantage of the function-driven approach is the potential for discovering entirely new classes of biocatalysts, with no similarity to known enzymes. However, the success of such screening relies on the kind of biotransformation process that should produce metabolites that can be easily detected by simple activity tests (such as reaction color) and on compatibility of the cloned genes with the transcription and translation machinery of the heterologous host, usually *Escherichia coli*. To overcome this limitation, a special fosmid vector was developed for the expression of metagenomic libraries from thermophiles. This vector, allowing the library to be constructed in *E. coli* and subsequently transferred to *Thermus thermophilus* (Angelov et al. 2009), could be of great help for the correct expression and folding of enzymes form hyperthermophilic microorganisms and the functional screening of metagenomic libraries from extreme environments.

Acknowledgements This work was supported by a grant from the Italian Space Agency "*Exobiology and extreme environments: from molecular chemistry to the biology of extremophiles*" contract n. 2014-026-R.0 and a grant from the Ministero dell'Università e della Ricerca Scientifica—Industrial Research Project "*Integrated agro-industrial chains with high energy efficiency for the*

development of eco-compatible processes of energy and biochemicals production from renewable sources and for the land valorization (Enerbio-Chem)" contract n. PON01_01966, funded in the frame of Operative National Programme Research and Competitiveness 2007–2013 D. D. Prot. n. 01/Ric. 18.1.2010.

References

Allen ET, Day AL (1935) Hot springs of the Yellowstone National Park

Amann RI, Ludwig W, Schleifer KH (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol Rev 59:143–169

Angelov A, Mientus M, Liebl S, Liebl W (2009) A two-host fosmid system for functional screening of (meta)genomic libraries from extreme thermophiles. Syst Appl Microbiol 32: 177–185. doi:10.1016/j.syapm.2008.01.003

Auchtung TA, Shyndriayeva G, Cavanaugh CM (2011) 16S rRNA phylogenetic analysis and quantification of Korarchaeota indigenous to the hot springs of Kamchatka, Russia. Extremophiles 15:105–116. doi:10.1007/s00792-010-0340-5

Barth TFW (1950) Volcanic geology, hot springs and geysers of Iceland. Carnegie Institution of Washington, Washington

Beam JP, Jay ZJ, Kozubal MA, Inskeep WP (2014) Niche specialization of novel Thaumarchaeota to oxic and hypoxic acidic geothermal springs of Yellowstone National Park. ISME J 8: 938–951. doi:10.1038/ismej.2013.193

Blank CE, Cady SL, Pace NR (2002) Microbial composition of near-boiling silica-depositing thermal springs throughout Yellowstone National Park. Appl Environ Microbiol 68: 5123–5135. doi:10.1128/AEM.68.10.5123-5135.2002

Blumer-Schuette SE, Kataeva I, Westpheling J, Adams MW, Kelly RM (2008) Extremely thermophilic microorganisms for biomass conversion: status and prospects. Curr Opin Biotechnol 19:210–217. doi:10.1016/j.copbio.2008.04.007

Bolduc B, Shaughnessy DP, Wolf YI, Koonin EV, Roberto FF, Young M (2012) Identification of novel positive-strand RNA viruses by metagenomic analysis of Archaea-dominated yellowstone hot springs. J Virol 86:5562–5573. doi:10.1128/JVI.07196-11

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics, btu170. doi:10.1093/bioinformatics/btu170

Briggs BR, Brodie EL, Tom LM, Dong H, Jiang H, Huang Q, Wang S, Hou W, Wu G, Huang L, Hedlund BP, Zhang C, Dijkstra P, Hungate BA (2014) Seasonal patterns in microbial communities inhabiting the hot springs of Tengchong, Yunnan Province, China. Environ Microbiol 16:1579–1591. doi:10.1111/1462-2920.12311

Brock TD (2001) The origins of research on Thermophiles. In: Reysenbach A-L, Voytek M, Mancinelli R (eds) Thermophiles biodiversity, ecology, and evolution. Springer, Boston, pp 1–9

Brock TD, Brock ML (1967) The hot springs of the Furnas Valley, Azores. Int Rev der gesamten Hydrobiol und Hydrogr 52:545–558. doi:10.1002/iroh.19670520405

Brock TD, Brock KM, Belly RT, Weiss RL (1972) Sulfolobus: a new genus of sulfur-oxidizing bacteria living at low pH and high temperature. Arch Mikrobiol 84:54–68

Caporaso JG, Bittinger K, Bushman FD, DeSantis TZ, Andersen GL, Knight R (2010a) PyNAST: a flexible tool for aligning sequences to a template alignment. Bioinformatics 26:266–267. doi:10.1093/bioinformatics/btp636

Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, Fierer N, Peña AG, Goodrich JK, Gordon JI, Huttley GA, Kelley ST, Knights D, Koenig JE, Ley RE, Lozupone CA, McDonald D, Muegge BD, Pirrung M, Reeder J, Sevinsky JR, Turnbaugh PJ, Walters WA, Widmann J, Yatsunenko T, Zaneveld J, Knight R (2010b) QIIME allows analysis of high-throughput community sequencing data. Nat Methods 7:335–336. doi:10.1038/nmeth.f.303

Chan CS, Chan K-G, Tay Y-L, Chua Y-H, Goh KM (2015) Diversity of thermophiles in a Malaysian hot spring determined using 16S rRNA and shotgun metagenome sequencing. Front Microbiol 6:177. doi:10.3389/fmicb.2015.00177

Chien A, Edgar DB, Trela JM (1976) Deoxyribonucleic acid polymerase from the extreme thermophile Thermus aquaticus. J Bacteriol 127:1550–1557

Cobucci-Ponzano B, Strazzulli A, Iacono R, Masturzo G, Giglio R, Rossi M, Moracci M (2015) Novel thermophilic hemicellulases for the conversion of lignocellulose for second generation biorefineries. Enzyme Microb Technol. doi:10.1016/j.enzmictec.2015.06.014

Cowan D, Ramond J-B, Makhalanyane T, De Maayer P (2015) Metagenomics of extreme environments. Curr Opin Microbiol 25:97–102. doi:10.1016/j.mib.2015.05.005

Eder W, Huber R (2002) New isolates and physiological properties of the Aquificales and description of Thermocrinis albus sp. nov. Extremophiles 6:309–318. doi:10.1007/s00792-001-0259-y

Elleuche S, Schäfers C, Blank S, Schröder C, Antranikian G (2015) Exploration of extremophiles for high temperature biotechnological processes. Curr Opin Microbiol 25:113–119. doi:10.1016/j.mib.2015.05.011

Ellis A, Mahon WA (1967) Natural hydrothermal systems and experimental hot water/rock interactions (Part II). Geochim Cosmochim Acta 31:519–538. doi:10.1016/0016-7037(67)90032-4

Eme L, Reigstad LJ, Spang A, Lanzén A, Weinmaier T, Rattei T, Schleper C, Brochier-Armanet C (2013) Metagenomics of kamchatkan hot spring filaments reveal two new major (hyper) thermophilic lineages related to thaumarchaeota. Res Microbiol 164:425–438. doi:10.1016/j.resmic.2013.02.006

Faith DP (1992) Conservation evaluation and phylogenetic diversity. Biol Conserv 61:1–10. doi:10.1016/0006-3207(92)91201-3

Fouke BW, Farmer JD, Des Marais DJ, Pratt L, Sturchio NC, Burns PC, Discipulo MK (2000) Depositional facies and aqueous-solid geochemistry of travertine-depositing hot springs (Angel Terrace, Mammoth Hot Springs, Yellowstone National Park, USA). J Sediment Res 70:565–585. doi:10.1306/2DC40929-0E47-11D7-8643000102C1865D

Fouke BW, Bonheyo GT, Sanzenbacher B, Frias-Lopez J (2003) Partitioning of bacterial communities between travertine depositional facies at Mammoth Hot Springs, Yellowstone National Park, USA. Can J Earth Sci 40:1531–1548

Graham JE, Clark ME, Nadler DC, Huffer S, Chokhawala HA, Rowland SE, Blanch HW, Clark DS, Robb FT (2011) Identification and characterization of a multidomain hyperthermophilic cellulase from an archaeal enrichment. Nat Commun 2:375. doi:10.1038/ncomms1373

Haas BJ, Gevers D, Earl AM, Feldgarden M, Ward DV, Giannoukos G, Ciulla D, Tabbaa D, Highlander SK, Sodergren E, Methé B, DeSantis TZ, Petrosino JF, Knight R, Birren BW (2011) Chimeric 16S rRNA sequence formation and detection in Sanger and 454-pyrosequenced PCR amplicons. Genome Res 21:494–504. doi:10.1101/gr.112730.110

Haki GD, Rakshit SK (2003) Developments in industrially important thermostable enzymes: a review. Bioresour Technol 89:17–34

Hall JR, Mitchell KR, Jackson-Weaver O, Kooser AS, Cron BR, Crossey LJ, Takacs-Vesbach CD (2008) Molecular characterization of the diversity and distribution of a thermal spring microbial community by using rRNA and metabolic genes. Appl Environ Microbiol 74:4910–4922. doi:10.1128/AEM.00233-08

Hamamura N, Macur RE, Korf S, Ackerman G, Taylor WP, Kozubal M, Reysenbach A-L, Inskeep WP (2009) Linking microbial oxidation of arsenic with detection and phylogenetic analysis of arsenite oxidase genes in diverse geothermal environments. Environ Microbiol 11:421–431. doi:10.1111/j.1462-2920.2008.01781.x

Hedenquist JW, Henley RW (1985) Hydrothermal eruptions in the Waiotapu geothermal system, New Zealand; their origin, associated breccias, and relation to precious metal mineralization. Econ Geol 80:1640–1668. doi:10.2113/gsecongeo.80.6.1640

Hetzer A, Morgan HW, McDonald IR, Daughney CJ (2007) Microbial life in Champagne Pool, a geothermal spring in Waiotapu, New Zealand. Extremophiles 11:605–614. doi:10.1007/s00792-007-0073-2

Hou W, Wang S, Dong H, Jiang H, Briggs BR, Peacock JP, Huang Q, Huang L, Wu G, Zhi X, Li W, Dodsworth JA, Hedlund BP, Zhang C, Hartnett HE, Dijkstra P, Hungate BA (2013) A comprehensive census of microbial diversity in hot springs of Tengchong, Yunnan Province China Using 16S rRNA Gene Pyrosequencing. PLoS ONE 8:e53350. doi:10.1371/journal.pone.0053350

Huang Q, Dong CZ, Dong RM, Jiang H, Wang S, Wang G, Fang B, Ding X, Niu L, Li X, Zhang C, Dong H (2011) Archaeal and bacterial diversity in hot springs on the Tibetan Plateau, China. Extremophiles 15:549–563. doi:10.1007/s00792-011-0386-z

Huber R, Huber H, Stetter KO (2000) Towards the ecology of hyperthermophiles: biotopes, new isolation strategies and novel metabolic properties. FEMS Microbiol Rev 24:615–623. doi:10.1016/S0168-6445(00)00049-8

Hug K, Maher WA, Stott MB, Krikowa F, Foster S, Moreau JW (2014) Microbial contributions to coupled arsenic and sulfur cycling in the acid-sulfide hot spring Champagne Pool, New Zealand. Front Microbiol 5:1–14. doi:10.3389/fmicb.2014.00569

Hugenholtz P, Pitulle C, Hershberger KL, Pace NR (1998) Novel division level bacterial diversity in a Yellowstone hot spring novel division level bacterial diversity in a Yellowstone hot spring. J Bacteriol 180:366–376

Huson DH, Weber N (2013) Microbial community analysis using MEGAN. Methods Enzymol 531:465–485. doi:10.1016/B978-0-12-407863-5.00021-6

Inskeep WP, Ackerman GG, Taylor WP, Kozubal M, Korf S, Macur RE (2005) On the energetics of chemolithotrophy in nonequilibrium systems: case studies of geothermal springs in Yellowstone National Park. Geobiology 3:297–317. doi:10.1111/j.1472-4669.2006.00059.x

Inskeep WP, Rusch DB, Jay ZJ, Herrgard MJ, Kozubal MA, Richardson TH, Macur RE, Hamamura N, de Jennings RM, Fouke BW, Reysenbach A-L, Roberto F, Young M, Schwartz A, Boyd ES, Badger JH, Mathur EJ, Ortmann AC, Bateson M, Geesey G, Frazier M (2010) Metagenomes from high-temperature chemotrophic systems reveal geochemical controls on microbial community structure and function. PLoS One 5:e9773. doi:10.1371/journal.pone.0009773

Inskeep WP, Jay ZJ, Herrgard MJ, Kozubal MA, Rusch DB, Tringe SG, Macur RE, Jennings RDM, Boyd ES, Spear JR, Roberto FF (2013a) Phylogenetic and functional analysis of metagenome sequence from high-temperature archaeal habitats demonstrate linkages between metabolic potential and geochemistry. Front Microbiol. doi:10.3389/fmicb.2013.00095

Inskeep WP, Jay ZJ, Tringe SG, Herrgård MJ, Rusch DB (2013b) The YNP metagenome project: environmental parameters responsible for microbial distribution in the yellowstone geothermal ecosystem. Front Microbiol 4:67. doi:10.3389/fmicb.2013.00067

Isaia R, Marianelli P, Sbrana A (2009) Caldera unrest prior to intense volcanism in Campi Flegrei (Italy) at 4.0 ka B.P.: implications for caldera dynamics and future eruptive scenarios. Geophys Res Lett 36:L21303. doi:10.1029/2009GL040513

Jay Z, Planer-Friedrich B, Rusch D, Inskeep W (2011) Linking geochemistry to microbial community structure and function in sulfidic geothermal systems of Yellowstone National Park. Mineral Mag 75:1104

Jiang H, Huang Q, Dong H, Wang P, Wang F, Li W, Zhang C (2010) RNA-based investigation of ammonia-oxidizing archaea in hot springs of Yunnan Province, China. Appl Environ Microbiol 76:4538–4541. doi:10.1128/AEM.00143-10

Jones B, Renaut R, Rosen M (2001) Biogenicity of gold- and silver-bearing siliceous sinters forming in hot (75 C°) anaerobic spring-waters of Champagne Pool, Waiotapu, North Island, New Zealand. J Geol Soc Lond 158:895–911. doi:10.1144/0016-764900-131

Ko M-S, Park H-S, Kim K-W, Lee J-U (2013) The role of Acidithiobacillus ferrooxidans and Acidithiobacillus thiooxidans in arsenic bioleaching from soil. Environ Geochem Health 35:727–733. doi:10.1007/s10653-013-9530-2

Kozubal M, Macur RE, Korf S, Taylor WP, Ackerman GG, Nagy A, Inskeep WP (2008) Isolation and distribution of a novel iron-oxidizing crenarchaeon from acidic geothermal springs in Yellowstone National Park. Appl Environ Microbiol 74:942–949. doi:10.1128/AEM.01200-07

Kozubal MA, Romine M, deM Jennings R, Jay ZJ, Tringe SG, Rusch DB, Beam JP, McCue LA, Inskeep WP (2013) Geoarchaeota: a new candidate phylum in the Archaea from high-temperature acidic iron mats in Yellowstone National Park. ISME J 7:622–634. doi:10.1038/ismej.2012.132

Kublanov IV, Perevalova AA, Slobodkina GB, Lebedinsky AV, Bidzhieva SK, Kolganova TV, Kaliberda EN, Rumsh LD, Haertlé T, Bonch-Osmolovskaya EA (2009) Biodiversity of thermophilic prokaryotes with hydrolytic activities in hot springs of Uzon Caldera, Kamchatka (Russia). Appl Environ Microbiol 75:286–291. doi:10.1128/AEM.00607-08

Lewin A, Wentzel A, Valla S (2013) Metagenomics of microbial life in extreme temperature environments. Curr Opin Biotechnol 24:516–525. doi:10.1016/j.copbio.2012.10.012

López-López O, Cerdán M, González-Siso M (2013) Hot spring metagenomics. Life 3:308–320. doi:10.3390/life3020308

López-López O, Cerdán ME, González Siso MI (2014) New extremophilic lipases and esterases from metagenomics. Curr Protein Pept Sci 15:445–455

Lorenz P, Liebeton K, Niehaus F, Eck J (2002) Screening for novel enzymes for biocatalytic processes: accessing the metagenome as a resource of novel functional sequence space. Curr Opin Biotechnol 13:572–577

Macur RE, Jay ZJ, Taylor WP, Kozubal MA, Kocar BD, Inskeep WP (2013) Microbial community structure and sulfur biogeochemistry in mildly-acidic sulfidic geothermal springs in Yellowstone National Park. Geobiology 11:86–99. doi:10.1111/gbi.12015

Marsh CL, Larsen DH (1953) Characterization of some thermophilic bacteria from the Hot Springs of Yellowstone National Park. J Bacteriol 65:193–197

Menzel P, Gudbergsdóttir SR, Rike AG, Lin L, Zhang Q, Contursi P, Moracci M, Kristjansson JK, Bolduc B, Gavrilov S, Ravin N, Mardanov A, Bonch-Osmolovskaya E, Young M, Krogh A, Peng X (2015) Comparative metagenomics of eight geographically remote terrestrial hot springs. Microb Ecol. doi:10.1007/s00248-015-0576-9

Meyer-Dombard D, Shock E, Amend J (2005) Archaeal and bacterial communities in geochemically diverse hot springs of Yellowstone National Park, USA. Geobiology 3: 211–227. doi:10.1111/j.1472-4669.2005.00052.x

Miller-Coleman RL, Dodsworth JA, Ross CA, Shock EL, Williams AJ, Hartnett HE, McDonald AI, Havig JR, Hedlund BP (2012) Korarchaeota diversity, biogeography, and abundance in Yellowstone and Great Basin hot springs and ecological niche modeling based on machine learning. PLoS ONE 7:e35964. doi:10.1371/journal.pone.0035964

Moser MJ, Di Francesco RA, Gowda K, Klingele AJ, Sugar DR, Stocki S, Mead DA, Schoenfeld TW (2012) Thermostable DNA polymerase from a viral metagenome is a potent RT-PCR enzyme. PLoS One. doi:10.1371/journal.pone.0038371

Mountain B, Benning L, Boerema J (2003) Experimental studies on New Zealand hot spring sinters: rates of growth and textural development. Can J Earth Sci 40:1643–1667. doi:10.1139/E03-068

Nakagawa S, Shtaih Z, Banta A, Beveridge TJ, Sako Y, Reysenbach A-L (2005) Sulfurihydrogenibium yellowstonense sp. nov., an extremely thermophilic, facultatively heterotrophic, sulfur-oxidizing bacterium from Yellowstone National Park, and emended descriptions of the genus Sulfurihydrogenibium, Sulfurihydrogenibium subterraneum. Int J Syst Evol Microbiol 55:2263–2268. doi:10.1099/ijs.0.63708-0

Olsen GJ, Woese CR, Overbeek R (1994) The winds of (evolutionary) change: breathing new life into microbiology. J Bacteriol 176:1–6

Orsi G, De Vita S, di Vito M (1996) The restless, resurgent Campi Flegrei nested caldera (Italy): constraints on its evolution and configuration. J Volcanol Geotherm Res 74:179–214. doi:10.1016/S0377-0273(96)00063-7

Páez-Espino D, Tamames J, de Lorenzo V, Cánovas D (2009) Microbial responses to environmental arsenic. Biometals 22:117–130. doi:10.1007/s10534-008-9195-y

Park CB, Lee SB (1999) Inhibitory effect of mineral ion accumulation on high density growth of the hyperthermophilic archaeon Sulfolobus solfataricus. J Biosci Bioeng 87:315–319

Petrosino S, Damiano N, Cusano P, Di Vito MA, de Vita S, Del Pezzo E (2012) Subsurface structure of the Solfatara volcano (Campi Flegrei caldera, Italy) as deduced from joint seismic-noise array, volcanological and morphostructural analysis. Geochem Geophys Geosyst 13:n/a–n/a. doi:10.1029/2011GC004030

Planer-Friedrich B, Franke D, Merkel B, Wallschläger D (2008) Acute toxicity of thioarsenates to Vibrio fischeri. Environ Toxicol Chem 27:2027–2035. doi:10.1897/07-633.1

Pride DT, Schoenfeld T (2008) Genome signature analysis of thermal virus metagenomes reveals Archaea and thermophilic signatures. BMC Genom 9:420. doi:10.1186/1471-2164-9-420

Pruesse E, Quast C, Knittel K, Fuchs BM, Ludwig W, Peplies J, Glöckner FO (2007) SILVA: a comprehensive online resource for quality checked and aligned ribosomal RNA sequence data compatible with ARB. Nucleic Acids Res 35:7188–7196. doi:10.1093/nar/gkm864

Reysenbach AL, Wickham GS, Pace NR (1994) Phylogenetic analysis of the hyperthermophilic pink filament community in Octopus Spring, Yellowstone National Park. Appl Environ Microbiol 60:2113–2119

Reysenbach AL, Ehringer M, Hershberger K (2000) Microbial diversity at 83 degrees C in Calcite Springs, Yellowstone National Park: another environment where the Aquificales and "Korarchaeota" coexist. Extremophiles 4:61–67

Reysenbach A-L, Banta A, Civello S, Daly J, Mitchel K, Ladonde S, Konhauserm K, Rodman A, Rusterholtz K, Takacs-Vesbach C (2005) Aquificales in Yellowstone National Park. In: Inskeep WP, Mcdermott TR (eds) Geothermal biology and geochemistry in YNP. Montana State University Publications, Bozeman, MT, pp 129–142

Reysenbach A-L, Hamamura N, Podar M, Griffiths E, Ferreira S, Hochstein R, Heidelberg J, Johnson J, Mead D, Pohorille A, Sarmiento M, Schweighofer K, Seshadri R, Voytek MA (2009) Complete and draft genome sequences of six members of the Aquificales. J Bacteriol 191:1992–1993. doi:10.1128/JB.01645-08

Rosi M, Santacroce R (1984) Volcanic hazard assessment in the Phlegraean Fields: a contribution based on stratigraphic and historical data. Bull Volcanol 47:359–370. doi:10.1007/BF01961567

Sahm K, John P, Nacke H, Wemheuer B, Grote R, Daniel R, Antranikian G (2013) High abundance of heterotrophic prokaryotes in hydrothermal springs of the Azores as revealed by a network of 16S rRNA gene-based methods. Extremophiles 17:649–662. doi:10.1007/s00792-013-0548-2

Schoenfeld T, Patterson M, Richardson PM, Wommack KE, Young M, Mead D (2008) Assembly of viral metagenomes from yellowstone hot springs. Appl Environ Microbiol 74:4164–4174. doi:10.1128/AEM.02598-07

Schröder C, Elleuche S, Blank S, Antranikian G (2014) Characterization of a heat-active archaeal β-glucosidase from a hydrothermal spring metagenome. Enzyme Microb Technol 57:48–54. doi:10.1016/j.enzmictec.2014.01.010

Sharma A, Kawarabayasi Y, Satyanarayana T (2012) Acidophilic bacteria and archaea: acid stable biocatalysts and their potential applications. Extremophiles 16:1–19. doi:10.1007/s00792-011-0402-3

Simon C, Wiezer A, Strittmatter AW, Daniel R (2009) Phylogenetic diversity and metabolic potential revealed in a glacier ice metagenome. Appl Environ Microbiol 75:7519–7526. doi:10.1128/AEM.00946-09

Sofía Urbieta M, Toril EG, Alejandra Giaveno M, Bazán AA, Donati ER (2014) Archaeal and bacterial diversity in five different hydrothermal ponds in the Copahue region in Argentina. Syst Appl Microbiol 37:429–441. doi:10.1016/j.syapm.2014.05.012

Song Z-Q, Chen J-Q, Jiang H-C, Zhou E-M, Tang S-K, Zhi X-Y, Zhang L-X, Zhang C-LL, Li W-J (2010) Diversity of Crenarchaeota in terrestrial hot springs in Tengchong, China. Extremophiles 14:287–296. doi:10.1007/s00792-010-0307-6

Stauder S, Raue B, Sacher F (2005) Thioarsenates in sulfidic waters. Environ Sci Technol 39:5933–5939. doi:10.1021/es048034k

Stetter KO (1996) Hyperthermophilic procaryotes. FEMS Microbiol Rev 18:149–158
Stetter KO, Fiala G, Huber G, Huber R, Segerer A (1990) Hyperthermophilic microorganisms. FEMS Microbiol Lett 75:117–124. doi:10.1111/j.1574-6968.1990.tb04089.x
Takacs-Vesbach C, Inskeep WP, Jay ZJ, Herrgard MJ, Rusch DB, Tringe SG, Kozubal MA, Hamamura N, Macur RE, Fouke BW, Reysenbach AL, McDermott TR, Jennings RDM, Hengartner NW, Xie G (2013) Metagenome sequence analysis of filamentous microbial communities obtained from geochemically distinct geothermal channels reveals specialization of three aquificales lineages. Front Microbiol. doi:10.3389/fmicb.2013.00084
Tirawongsaroj P, Sriprang R, Harnpicharnchai P, Thongaram T, Champreda V, Tanapongpipat S, Pootanakit K, Eurwilaichitr L (2008) Novel thermophilic and thermostable lipolytic enzymes from a Thailand hot spring metagenomic library. J Biotechnol 133:42–49. doi:10.1016/j.jbiotec.2007.08.046
Troiano A, Di Giuseppe MG, Patella D, Troise C, De Natale G (2014) Electromagnetic outline of the solfatara-pisciarelli hydrothermal system, Campi Flegrei (Southern Italy). J Volcanol Geotherm Res 277:9–21. doi:10.1016/j.jvolgeores.2014.03.005
Ullrich MK, Pope JG, Seward TM, Wilson N, Planer-Friedrich B (2013) Sulfur redox chemistry governs diurnal antimony and arsenic cycles at Champagne Pool, Waiotapu, New Zealand. J Volcanol Geotherm Res 262:164–177. doi:10.1016/j.jvolgeores.2013.07.007
Vick TJ, Dodsworth JA, Costa KC, Shock EL, Hedlund BP (2010) Microbiology and geochemistry of Little Hot Creek, a hot spring environment in the Long Valley Caldera. Geobiology 8:140–154. doi:10.1111/j.1472-4669.2009.00228.x
Wang S, Dong H, Hou W, Jiang H, Huang Q, Briggs BR, Huang L (2014) Greater temporal changes of sediment microbial community than its waterborne counterpart in Tengchong hot springs, Yunnan Province, China. Sci Rep 4:7479. doi:10.1038/srep07479
Wemheuer B, Taube R, Akyol P, Wemheuer F, Daniel R (2013) Microbial diversity and biochemical potential encoded by thermal spring metagenomes derived from the Kamchatka peninsula. Archaea. doi:10.1155/2013/136714
Woese CR, Kandler O, Wheelis ML (1990) Towards a natural system of organisms: proposal for the domains Archaea, Bacteria, and Eucarya. Proc Natl Acad Sci USA 87:4576–4579
Yamamoto H, Hiraishi A, Kato K, Chiura HX, Maki Y, Shimizu A (1998) Phylogenetic evidence for the existence of novel thermophilic bacteria in hot spring sulfur-turf microbial mats in Japan. Appl Environ Microbiol 64:1680–1687
Yang T, Lyons S, Aguilar C, Cuhel R, Teske A (2011) Microbial communities and chemosynthesis in Yellowstone Lake sublacustrine hydrothermal vent waters. Front Microbiol 2:130. doi:10.3389/fmicb.2011.00130
Young M, Wiedenheft B, Snyder J, Spuhler J, Roberto F, Douglas T (2005) Archeal viruses from Yellowstone's high temperature environments. In: Inskeep WP, Mcdermott TR (eds) Geothermal biology and geochemistry in YNP. Mountana State University Publications, Bozeman, MT, pp 289–304
Zillig W, Stetter KO, Wunderl S, Schulz W, Priess H, Scholz I (1980) The Sulfolobus-"Caldariella" group: taxonomy on the basis of the structure of DNA-dependent RNA polymerases. Arch Microbiol 125:259–269. doi:10.1007/BF00446886