9

Metagenomic Insights into Microbial Diversity and Metabolic Potential of Hot Spring Ecosystems

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Abstract

Hot water springs or hydrothermal springs are places where warm or hot water comes out of earth surfaces regularly or for a significant period, in a year. These ecosystems present an epitome of extreme environments and are extensively distributed all over the globe. Geographically, these ecosystems encompass unique physical and chemical characteristics. Interestingly, 16S rRNA gene analysis in combination with next-generation sequencing has provided in-depth knowledge about phylogeny and the metabolic potential of a particular environment, including the hot springs. Every hot spring is unique and dynamic in its characteristics compare to the other. Investigation of metagenome from diverse ecological habitats, using high-throughput sequencing or library construction, has led to the discovery of a number of novel biocatalysts. Metagenomic studies in recent years have achieved two major goals: first it has resulted in deep understanding about structural and functional dynamics of microbial communities, and secondly, it has led to the discovery of diverse novel bioactive molecules. This book chapter will shed light into the role of metagenome gene cloning in revealing the true and comprehensive diversity and the metabolic potential of microbes in hot spring ecosystems.

Keywords

Metagenomics • Hot spring • Bacterial phylogeny • Ecosystems • 16S rRNA

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9.1 Introduction

The approximate number of prokaryotic cells in biosphere may exceed ~4–6 × 10^{30} (Whitman et al. 1998). The major fractions of prokaryotic organisms yet remain unexplored, and thus it presents an exciting challenge for scientific community to explore the genetic and metabolic diversity from various ecosystems. Investigation and analysis of 16S rRNA genes from diverse ecological habitats have demonstrated the presence of novel sequences with unique origin (Felske et al. 1999). It was established in a number of studies that 90–95% bacteria remain uncultured (Amann et al. 1995; Hugenholtz et al. 1998a; Hugenholtz 2002). The term metagenomics was coined by Jo Handelsman (Handelsman et al. 1998) and refers to genomic analysis of environmental DNA. Hot water springs or hydrothermal springs are the places where warm or hot groundwater comes out of earth surface regularly or for a significant period, in a year. These ecosystems present an epitome of extreme environment and are extensively distributed all over the world and are quite distinct with respect to their physicochemical characteristics (Hugenholtz et al. 1998b; Marteinsson et al. 2001).

These ecosystems hold variety of microflora with vast gene pool which can be explored for biotechnological applications. Metagenomic studies thus offer major facade for microbiologists, to connect phylogeny with ecological functions (Sharma et al. 2005).

Microbial community structure in hot spring is strongly dependent on the gradients of temperature, ecological interactions, chemistry of the underlying rocks, pH, oxidation-reduction potential or concentrations of various dissolved sulphides and inorganic carbons (Fouke et al. 2000; Dick and Shock 2013). Microorganisms possess propensity to append and aggregate to surfaces, when they come under the vicinity of water that results in formation of intricate networks (Gerbersdorf and Wieprecht 2015). Hot spring ecosystem holds enormous microbial diversity capable of surviving and blossom under array of environmental conditions (Wang et al. 2013; Chan et al. 2015). However, the range of mesophilic or thermophilic diversity in these ecosystems, as discussed above, is strongly dependent upon the temperature (Hobel et al. 2005). Construction and functional screening of metagenomic library from such ecosystems have already provided access to untapped wealth of active molecules (Simon et al. 2009; Xie et al. 2011; Tirawongsaroj et al. 2008; Steele et al. 2009; Jimenez et al. 2012a, b). Altogether, in recent years, culture-independent studies have achieved two major goals: firstly, it has enabled researchers in better understanding of structure and functioning of the microbes, and secondly, it has resulted in identification of novel active biomolecules (Neelakanta and Sultana 2013).

9.2 Microbial Diversity in Hot Springs

The major hot spring in the hot springs are found in Canada, New Zealand, United States, Chile, Japan, India and Malaysia (Song et al. 2010). India is referred to be one of the most tectonically active areas in the world, and according to geological surveys, it harbours ~340 hot water springs, which are classified into six geothermal provinces (Bisht et al. 2011). Here, in this book chapter, insights would be provided into the latest study being carried out to investigate microbial diversity and the metabolic potential of hot spring ecosystems. Starting with Indian subcontinents, a hot spring analysed in West Bengal predominantly demonstrated the presence of *Proteobacteria* and *Cyanobacteria* (Ghosh et al. 2003). 16S rRNA investigation of two hot springs, Tulsi Shyam and Lasundra of Gujarat state, in India, employing tag-encoded FLX amplicon pyrosequencing (bTEFAP) and shotgun sequencing approach, respectively, revealed variation in distribution of microbial diversity in these two hot springs, whereas Tulsi Shyam hot spring predominantly revealed the presence of *Firmicutes* (65.38%), *Proteobacteria* (21.21%) and an unclassified bacterial population ~10.69% (Ghelani et al. 2015). High-throughput sequencing of community DNA from Lasundra hot spring using an Ion Torrent PGM platform revealed predominantly *Bacillus* (86.7%), *Geobacillus* (2.4%) and *Paenibacillus* (1.0%) (Mangrola et al. 2015). Hot springs of Tibetan Plateau investigated employing Cluster and SIMPER divulges that temperature can greatly affect all over distribution of microbial diversity. Analysis of microbial distribution pattern using non-metric multidimensional scaling (NMDS) and principal coordinates analysis (PCoA) at species-level OTUs depicted a total of 42 bacterial phyla. Temperature range of 66–75 °C supported *Aquificae*, *Archaea* and GAL35 (a novel bacterial lineage), whose abundance exhibited a positive correlation with increasing temperature. In sharp contrast to this, *Deinococcus-Thermus*, *Cyanobacteria* and *Chloroflexi* showed its predominance in the temperature range 22–60 °C and were negatively associated with the temperature (Wang et al. 2013). Investigation of microbial diversity from Yellowstone National Park (YNP), United States, revealed varied composition of thermophilic microorganisms. Analysis of 16S rRNA genes from metagenome sample derived from 19 positions of 11 geothermal springs of YNP showed foremost presence of *Metallosphaera* and *Sulfolobus*. The bacterial genus *Hydrogenobaculum* showed its marked presence, followed by *Acidimicrobium*, *Acidovorax*, *Acidicaldus*, *Methylacidiphilum*, *Meiothermus*, *Geothermobacterium* and *Sulfobacillus*. Interestingly, four novel lineages that represented *Sulfolobus*, *Sulfolobales*, *Sulfobacillus* and *Acidicaldus* revealed maximum similarity to *Sulfolobus* sp. T1 (96.6%), *Sulfolobus islandicus* (88%), *Sulfobacillus acidophilus* (98%) and *Acidicaldus organivorans* (97.6%), respectively (Kozubal et al. 2012).

Interestingly, several hot springs characterized from YNP portrayed the dominant existence of photosynthetic microorganisms. A specific example is the mushroom spring, where four members of the bacterial community, *Cyanobacteria*, *Chloroflexi*, *Chlorobi* and *Acidobacteria*, showed their predominant occurrence in a phototrophic mat community (Liu et al. 2011). Furthermore, 16S rRNA and RFLP analysis of metagenome sample derived from three hot springs of Indonesia showed presence of *Proteobacteria*, *Bacillus* and *Flavobacterium*; interestingly, all these bacterial groups are not usually related with the thermophilic lineages (Baker et al. 2001). The site near volcanic eruption offers opportunity to explore, understand and compare the structural and functional dynamics of extremophilic archaea. Analysis of microbial communities from two such extreme sites (Mutnovsky and Uzon) of Kamchatka Peninsula revealed the presence of notable members from various communities. Interestingly, the phylum *Thaumarchaeota* comprises 57% of the total community at Mutnovsky, whereas it constituted 68% of the total community at Uzon sample, and members of phylum *Euryarchaeota* dominated the Mutnovsky by 34.7%. Among the bacterial lineages, *Thermotogae* showed its abundant presence in Mutnovsky, whereas it was negligible in Uzon. In sharp contrast to this, *Proteobacteria* followed by *Enterobacteriaceae*, *Aquificae* and *Thermodesulfobacteria* showed their marked presence at Uzon (Wemheuer et al. 2013). Yet in another study, 16S rRNA gene sequencing, in combination with the next-generation sequencing of metagenome from the hot spring of Sungai Klah, Malaysia, revealed foremost presence of *Firmicutes* (37.15%) and *Proteobacteria* (19.26%), whereas *Aquificae*, *Verrucomicrobia*, *Thermotogae* and 29 other members demonstrated less abundance. Notably, the study reports the presence of several phototrophic bacteria like *Roseiflexus*, *Porphyrobacter* and *Chloroflexus*. In addition, various pathogenic microbes like *Clostridium hiranonis*, *Brucella suis*, *Legionella pneumophila*, *Leptospira licerasiae*, *Leptospira wolffii*, *Pseudomonas fluorescens*, *Rickettsia montanensis*, *Rickettsiales*, etc. were also observed. Nevertheless, several microorganisms involved in carbon, sulphur and nitrogen metabolism were also found in this ecosystem (Chan et al. 2015). Microbial phylogeny investigation of hot springs from China revealed the presence of distinct monophyletic bacterial groups and several unidentified lineages. The archaea identified from this hot spring belong to *Euryarchaeota*, *Crenarchaeota* and *Korarchaeota* (Pagaling et al. 2012). The group *Crenarchaeota* is suggested to play a major role in the nitrification process during the nitrogen cycle (Leininger et al. 2006; Reigstad et al. 2008). Metagenome investigation of thermal spring from South Africa showed dominant presence of genera *Stenotrophomonas*, *Hydrogenophaga*, *Flectobacillus*, *Rheinheimera*, *Pseudomonas*, *Zavarzinella*, *Aquaspirillum* and *Limnobacter* (Tekere et al. 2015).

In another study, community analysis of environmental DNA from three hot springs, Tshipise, Mphephu and Sagole of South Africa, demonstrated the dominant presence of *Bacteroidetes* and *Proteobacteria* in Mphephu. In contrast, *Proteobacteria* and *Cyanobacteria* showed their prominent occurrence in Tshipise and Sagole. Several other phyla recovered revealed their presence <0.20%. (Tekere

Table 9.1 Major representative phyla having physiological roles in hot springs

Organism	Functions	Physiological type	References
Chloroflexi (e.g. *Chloronema*)	BChl-c and BChl-a biosynthesis, oxidize sulphide to polysulphides	Chlorophototrophs	Bryant et al. (2012), Klatt et al. (2011)
Aquificae (e.g. *Aquificales*)	Biological oxidation of sulphur compounds	Autotrophs	Skirnisdottir et al. (2000)
Proteobacteria (e.g. *Thiobacillus*)	Calvin-Benson cycle, reductive tricarboxylic cycle	Aerobic chemoorganotrophs	Chan et al. (2015)
Cyanobacteria (e.g. *Synechococcus*)	Oxygenic photosynthesis, nitrogen metabolism	Oxygenic phototrophs	Steunou et al. (2006), Bhaya et al. (2007)
Planctomycetes	Reductive acetyl-CoA pathway	Oligotrophic aerobic chemoorganotroph	Chan et al. (2015)
Euryarchaeota (e.g. *Methanobacteriales*)	Methanogenesis, reductive acetyl-CoA pathway	–	Ward et al. (1998), Chan et al. (2015)
Thaumarchaeota (e.g. *Nitrososphaera*)	Oxidation of ammonia to nitrite in the nitrogen cycle	Chemolithoautotrophs	Reigstad et al. (2008)
Crenarchaeota (e.g. *Desulfurococcales*)	Nitrification, carbon fixation, sulphur respiration	Organotrophs, chemolithotrophs	Huber and Stetter (2001), Leininger et al. (2006)

et al. 2012). Altogether, it was observed that various hot springs investigated for the microbial diversity all over the world demonstrated significant level of variations in distribution pattern of microbial community. Table 9.1 further reports microbial diversity from few more hot springs explored recently.

9.3 Metabolic Potential of Hot Spring Environments

With the advent of next-generation sequencing (NGS), it has become possible to determine the metabolic potential of any microbiome. NGS investigation of metagenome and metatranscriptome from hot springs provides inventory of microbial communities inhabiting in such habitats.

A classic example is the investigation of hot spring from Shi-Huang-ping from Taiwan that showed abundant presence of *Hydrogenobaculum* as a principle microorganism in this hot spring. This study also demonstrated the presence of genes related to carbon assimilation, nitrogen fixation and sulphur and hydrogen metabolism (Lin et al. 2015). Investigation of metabolic and functional potential of mushroom and octopus thermal springs from Yellowstone National Park (YNP), along with numerous other hot springs, revealed that almost all microbes principally transcribed genes for chlorophototrophy (Klatt et al. 2011). Furthermore, a comparative study of phototrophic, streamer and archaeal communities from 20 geothermal areas of YNP demonstrated variations in the numerous functional categories, like cell replication, energy metabolism, nitrogen fixation, cofactor biosynthesis, fatty acid biosynthesis, nitrogen metabolism, amino acid biosynthesis, etc. (William et al. 2013). Comparative genomics of microbes from alkaline hot springs revealed presence of 3-hydroxypropionate autotrophic pathway in bacteria. These microbial mats present archetype for studying microbial community ecology in siliceous hot springs of YNP. Interestingly, molecular and microscopic analysis of microbial mats established the dominant presence of unicellular *Synechococcus* species and filamentous anoxygenic phototrophs (FAPs). This study further signifies that there is cross feeding of metabolites among different organisms (Klatt et al. 2007). Functional analysis of Lasundra hot spring from Gujarat, India, revealed the presence of several genes that participate in the metabolism of aromatic compounds (Mangrola et al. 2015).

Understanding biological processes that involve nitrification and ammonia oxidation can enhance our understanding about the biogeochemical nitrogen cycling. Initially, these processes were thought to be restricted to few bacterial groups of *Proteobacteria* (Purkhold et al. 2000). Recent development in the molecular biology, however, has depicted that archaea are also efficient in oxidation of ammonia into nitrite (Dodsworth et al. 2011). Interestingly, archaea now have been implicated dominant component of the ammonia oxidation in terrestrial and marine environments. In this context, a study conducted from 22 hot springs showed that out of 22 hot spring, only 14 showed positive ammonia monooxygenase gene (AMO) from terrestrial hot springs, and most of these genes were observed at temperature range of 82–97 °C and pH range of 2.5–7 (Reigstad et al. 2008). KEGG analysis of enzymes involved in different metabolic pathways from acidic hot spring of Colombian Andean region elucidated several genes that encode enzymes responsible for nitrogen and sulphur cycle (Jimenez et al. 2012a, b). Several studies correlate geochemistry with the microbiological processes (Vick et al. 2010; Swingley et al. 2012).

Microbes have been recognized as major source of bioactive compounds (Bottone and Peluso 2003; Volk 2006; Volk and Furkert 2006; Williams 2009). Microbial mats from hot spring environment have received much attention, due to their vast potential towards synthesis of novel bioactive compounds. In this context, investigation of antimicrobial potential of cyanobacterial mats was evaluated using direct microscopy, from four hot springs located in the Sultanate of Oman. Active components extracted resulted in isolation and identification of 74 chemical compounds that displayed inhibitory activities against a diverse range of bacterial species and a diatom *Amphora coffeaeformis*. Determination of bacterial community composition showed that cyanobacterial species identified has shared homology mainly with *Chroococcus*, *Phormidium*, *Leptolyngbya*, *Spirulina* and *Lyngbya* (Dobretsov et al. 2011). The metabolic potential of microorganisms associated with various metabolic pathways is further enlisted in Table 9.2.

Table 9.2 List of metabolic functions performed by microbes dominated in hot springs

Metabolic function	Bacteria	References
Carbon cycle		
Reductive citrate cycle	*Hydrogenobaculum*	Lin et al. (2015)
Hydroxypropionate-hydroxybutyrate cycle	*Sulfolobus*	Alber et al. (2006)
	Metallosphaera	Alber et al. (2006)
Reductive citrate cycle and dicarboxylate-hydroxybutyrate cycle	*T. uzoniensis*, *T. tenax*	Mardanov et al. (2011), Siebers et al. (2011)
Calvin cycle	*Acidithiobacillus*	You et al. (2011)
	Thiomonas	Duquesne et al. (2008)
Nitrogen cycle		
Fixation of nitrogen	*A. ferrooxidans*	Lin et al. (2015)
Transformation of nitroalkane compounds (R-NO_2) to nitrite	*Hydrogenobaculum*, *A. ferrooxidans*, *Thiomonas*	Lin et al. (2015)
Assimilatory nitrate reduction	*Sphingomonas* sp., *Candidatus*, *Koribacter versatilis*, *Acidobacterium capsulatum*, *Pseudochlorella* sp., *Thalassiosira pseudonana*, *Chthoniobacter flavus*	Jimenez et al. (2012a, b)
Sulphur metabolism		
Transformation of trithionate into sulphite with sulphite reductase	*Vulcanisaeta archaea*, *Thermoproteus tenax*, *Caldivirga maquilingensis*	Lin et al. (2015)
Conversion of thiosulphate into sulphate	*Thiomonas*	Lin et al. (2015)
Conversion of tetrathionate or trithionate into thiosulphate	*Hydrogenobaculum*, *S. tokodaii*, *Metallosphaera*	Auernik and Kelly (2008), Lin et al. (2015)
Sulphur oxidation	*Phaeodactylum tricornutum*	Jimenez et al. (2012a, b)
Sulphate reduction	*Pyrobaculum* spp. *Caldivirga* spp.	William et al. (2010)
	Thermodesulfovibrio yellowstonii	Henry et al. (1994)
	Thermodesulfovibrio aggregans	Sekiguchi et al. (2008)
	Desulfomicrobium thermophilum	Thevenieau et al. (2007)
	Desulfotomaculum carboxydivorans	Parshina et al. (2005)
	Desulfotomaculum kuznetsovii	Visser et al. (2013)
	Thermodesulfatator indicus	Moussard et al. (2004)
	Thermodesulfobacterium commune	Zeikus et al. (1983)
	Thermodesulfobium narugense	Mori et al. (2003)
	Archaeoglobus veneficus	Huber et al. (1997)
	Caldivirga maquilingensis	Itoh et al. (1999)
Reduction of sulphur to hydrogen sulphide	*Hippea maritima*	Miroshnichenko et al. (1999)
	Thermococcus gammatolerans	Jolivet et al. (2003)
	Thermofilum pendens	Anderson et al. (2008)
	Caldivirga maquilingensis	Itoh et al. (1999)
	Vulcanisaeta distributa	Itoh et al. (2002)
	Vulcanisaeta moutnovskia	Gumerov et al. (2011)

(continued)

Table 9.2 (continued)

Metabolic function	Bacteria	References
Iron metabolism		
Oxidation of iron	*Metallosphaera yellowstonensis*	Kozubal et al. (2011)
Reduction of ferric iron under anaerobic conditions	*Sulfolobales* str. MK5, *Acidicaldus* str. MK6	Kozubal et al. (2012)

9.4 Insights into Carbon, Nitrogen and Sulphur Cycle

The investigation of the major elemental cycles can help in predicting diverse microbial functions. The following paragraphs provide insights into various studies that report the role of metagenomics in understanding such processes.

9.5 Carbon Cycle

To date, several carbon assimilation pathways have been identified employing metagenomic studies. Reductive citrate cycle in *Hydrogenobaculum* was reported from hot springs of Taiwan (Lin et al. 2015). Microbial species *Sulfolobus* and *Metallosphaera* were reported to harbour genes that participate in hydroxypropionate-hydroxybutyrate cycle (Alber et al. 2006; Teufel et al. 2009). Interestingly, both reductive citrate cycle and dicarboxylate-hydroxybutyrate cycle take place in *T. uzoniensis* and *T. tenax* (Mardanov et al. 2011; Siebers et al. 2011). The presence of genes related to Calvin cycle in *Acidithiobacillus* and *Thiomonas* indicates that these microbes are dynamically involved in carbon metabolism (Duquesne et al. 2008; You et al. 2011).

9.6 Nitrogen Cycle

Nitrogen metabolism can provide insights about the biotransformation of various nitrogenous compounds. The processes like nitrogen fixation are well studied in *A. ferrooxidans*. Biological transformation of nitroalkane compounds to nitrite has been notably found in *Hydrogenobaculum*, *A. ferrooxidans* and *Thiomonas* (Lin et al. 2015). Major genes involved in nitrogen cycle include narG, narH, narI, norB, norE, norC, nifD, nifK, nirB, nirA and nirS encoding different enzymes. Importantly, microorganisms involved in the nitrogen metabolism belong to *Proteobacteria*, *Acidobacteria*, *Firmicutes*, *Nitrospira*, *Spartobacteria*, *Trebouxiophyceae*, *Coscinodiscophyceae*, etc. in acidic hot spring of Colombian Andes (Jimenez et al. 2012a, b).

9.7 Sulphur Metabolism

In addition to carbon and nitrogen metabolism, the presence of sulphur-metabolizing enzymes has been identified and mapped in various hot springs. Dominant microorganism involved in sulphur oxidation is *Phaeodactylum tricornutum* (Jimenez et al. 2012a, b). Several microbes have revealed the presence of key enzymes involved in sulphate as well as sulphur reduction. Other sulphur-related metabolic pathways that involve transformation of trithionate into sulphite are reported in *Vulcanisaeta*, *Thermoproteus* and *Caldivirga*. Furthermore, *Thiomonas* has key enzymes for converting thiosulphate into sulphate (Lin et al. 2015).

9.8 Biocatalysts Isolated from Hot Springs

Microorganisms from thermophilic environments are the major source of thermostable enzymes. *Taq* polymerase, the first enzyme isolated from the thermophilic strain *Thermus aquaticus*, has been an innovation towards the discovery of polymerase chain reaction (Chien et al. 1976). Enzymes obtained from these microorganisms have great potential to be used as biocatalysts for biotechnology and industrial purposes. With the increasing demand of thermostable enzyme in various chemical industries, their recovery from hot springs has increased tremendously. Thermostable enzymes have been extensively used in food, pharmaceuticals, cosmetics, geochemicals and leather, dairy, pulp and paper industries and for brewing and baking purposes (Haki and Rakshit 2003).

Various hot springs have been explored to obtain novel thermostable enzymes, e.g. investigation of metagenome from Lobios hot spring revealed the presence of 11 ORFs homologous to lipolytic enzymes. The enzyme showed sequence similarity to β-lactamase irrespective of showing any β-lactamase activity (López-López et al. 2015). Several other esterases were isolated from several hot springs all over the world (Rhee et al. 2005; Tirawongsaroj et al. 2008; Leis et al. 2015).

PCR-based cloning has also been successfully used in screening the novel enzymes directly from the metagenomic samples (Lorenz et al. 2002). A novel cyclomaltodextrinase gene was cloned from environmental DNA that has the ability to hydrolyse cyclodextrins and starch (Tang et al. 2006).

A gene encoding lipase enzyme was cloned from metagenome sample of Manikaran Sahib (Himachal Pradesh). Sequence analysis of the cloned gene revealed its identity with lipase gene of *Geobacillus*. Biochemical analysis of the lipase demonstrated its maximum activity at 60 °C (Sharma et al. 2011). Interestingly, the thermostability of the enzyme was further enhanced employing directed evolution (Sharma et al. 2012). Recently, thermostable protease isolated from *Bacillus licheniformis* of Unnai hot springs has been employed in various industrial settings (Dudhagara et al. 2014).

Recent studies from hot springs of Manikaran reported isolation of a *Bacillus altitudinis* IARI-MB-9 and *Gulbenkiania mobilis* IARI-MB-18 which produces

Table 9.3 List of enzymes isolated from hot springs

Enzyme	Source	Optimum temperature (°C)	Optimum pH	References
Taq Polymerase	Yellowstone National Park, USA	80	7.8	Chien et al. (1976)
Thermoalkaliphilic lipases	Hot springs of Southern Sinai	60	10	Deyaa et al. (2016)
Lipase	Hot springs of Manikaran	50	9.0	Sharma et al. (2011)
Esterase (Est1)	Hot springs in Tangkuban Perahu	90	6.0	Rhee et al. (2005)
Patatin-like phospholipase (PLP)	Thailand hot spring	70	9.0	Tirawongsaroj et al. (2008)
Esterase (Est1)	Thailand hot spring	70	9.0	Tirawongsaroj et al. (2008)
α-Amylase	Omer hot spring, Afyonkarahisar in Turkey	80	5.0	Ozdemir et al. (2015)
α-Amylase	Hot spring of Larijan, Iran	80	5.0–7.0	Mollania et al. (2009)
Amylase	Hot spring at Purwokerto, Central Java Province, Indonesia	60	8.0	Amin and Zusfahair (2012)
α-Amylase	Hot spring sources in Yangmingshan National Park, Northern Taiwan	70	5.5–6.5	Shaw et al. (1995)
Amylase	Wondo Genet hot spring	75–80	5.5	Mamo and Gessese (1999)
Lipase	Hot springs in Indonesia	50	7.5	Lee et al. (1999)
GH5 cellulase	Hot spring in Grensdalur, Iceland	70	5	Zarafeta et al. (2016)
Cellulase (EBI-244)	Great Boiling Spring, Gerlach, Nevada	109	–	Graham et al. (2011)
Cyclomaltodextrinase	Bor Khleung hot spring in Ratchaburi province, Thailand	50–55	6–7	Tang et al. (2006)
Xylanase	Hot spring in Yongtai (Fuzhou, China)	75	8.2	Liu et al. (2012)
Neopullulanase-like enzyme (Env Npu193A)	Bor Khleung hot spring in Thailand	75	7.0	Tang et al. (2008)
Alkaline Protease	Unnai hot spring	50	9.0	Dudhagara et al. (2015)
β-D-galactosidase (MbgI)	Geothermal springs in Northern Himalayan Region of India	65	8.0	Gupta et al. (2012)

Table 9.3 (continued)

Enzyme	Source	Optimum temperature (°C)	Optimum pH	References
Esterase (EstA2)	Hot springs in the town of Furnas, Azores, Portugal	80	8.0	Leis et al. (2015)
Esterase (EstB1)	Hot springs in the town of Furnas, Azores, Portugal	75	8.0	Leis et al. (2015)
Esterase (LOB4Est)	Lobios hot spring, in Ourense (Galician region, Spain)	40	7.5	López-López et al. (2015)
Lipolytic enzyme (PlpBW1)	Hot springs located in Kamchatka Peninsula	85	10	Wemheuer et al. (2013)
Lipolytic enzyme (EstBW1)	Hot springs located in Kamchatka Peninsula	90	7.0	Wemheuer et al. (2013)
Lipolytic enzyme (EstBW2)	Hot springs located in Kamchatka Peninsula	65	7.0	Wemheuer et al. (2013)

thermostable hydrolytic enzymes like CMCase, Xylanase, FPase and Cellobiose that display activities at high temperatures (Verma et al. 2015). In addition, various metagenomic studies also report isolation of two lipolytic enzymes—phospholipase and esterase from hot spring of Thailand (Thevenieau et al. 2007). Metagenome investigation of two hot springs from Kamchatka Peninsula resulted in isolation of novel genes encoding lipolytic and proteolytic enzymes that displayed maximum activities at 85, 90 and 65 °C, respectively (Wemheuer et al. 2013). Table 9.3 further enlists various enzymes reported from various hot spring ecosystems.

Conclusion

The biological diversity of bacteria can help us recognize the way of shaping and survival of these microbes in hot springs through various physicochemical conditions and biological interactions. The existence of biotechnological significant species in the metagenome suggests the impending applications of the hot spring bacteria that evoke the continuing research in this field.

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