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ORIGINAL PAPER

Analysis of the metatranscriptome of microbial communities of an alkaline hot sulfur spring revealed different gene encoding pathway enzymes associated with energy metabolism

Swetaleena Tripathy[1] · Soumesh Kumar Padhi[1] · Sriprakash Mohanty[1] · Mrinal Samanta[1] · Nikhil Kumar Maiti[1]

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Abstract Alkaline sulfur hot springs notable for their specialized and complex ecosystem powered by geothermal energy are abundantly rich in different chemotrophic and phototrophic thermophilic microorganisms. Survival and adaptation of these organisms in the extreme environment is specifically related to energy metabolism. To gain a better understanding of survival mechanism of the organisms in these ecosystems, we determined the different gene encoding enzymes associated with anaerobic pathways of energy metabolism by applying the metatranscriptomics approach. The analysis of the microbial population of hot sulfur spring revealed the presence of both aerobic and anaerobic organisms indicating dual mode of lifestyle of the community members. *Proteobacteria* (28.1 %) was the most dominant community. A total of 988 reads were associated with energy metabolism, out of which 33.7 % of the reads were assigned to nitrogen, sulfur, and methane metabolism based on KEGG classification. The major lineages of hot spring communities were linked with the anaerobic pathways. Different gene encoding enzymes (*hao*, *nir*, *nar*, *cysH*, *cysI*, *acs*) showed the involvement of microbial members in nitrification, denitrification, dissimilatory sulfate reduction, and methane generation. This study enhances our understanding of important gene encoding enzymes involved in energy metabolism, required for the survival and adaptation of microbial communities in the hot spring.

Keywords Hot spring · Microbial communities · Functional pathways · Energy metabolism

Abbreviations

mRNA	Messenger ribonucleic acid
cDNA	Complementary DNA
NGS	Next-generation sequencing
MG-RAST	Metagenomics-Rapid Annotation using Subsystem Technology
MEGAN	MEtaGenome ANalyzer

Introduction

Thermal springs are considered to be the crucial component of the chemosynthetic ecosystem, geared up by geothermal energy. They serve as the natural habitat of diversified microorganisms forming the lower most base of an aquatic food chain. Survival and adaptation of the microbial life in the thermal springs is generally dependent upon different metabolic pathways required for driving physiologically important processes. This, in particular, has been reflected in many studies based on the functional analysis of the microbial activities in the hot springs (Inskeep et al. 2010; Jimenez et al. 2012).

Hot sulfur springs with alkaline pH are rare to find in the geothermal areas of the world (Skirnisdottir et al. 2000). Besides the Icelandic hot springs, these types of springs are commonly found in the Eastern Ghats regions of India. The hot sulfur spring of Attri confined along the periphery of

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✉ Nikhil Kumar Maiti
maitink@yahoo.co.in

[1] Environmental Microbiology Unit, Division of Fish Health Management, Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar 751002, Odisha, India

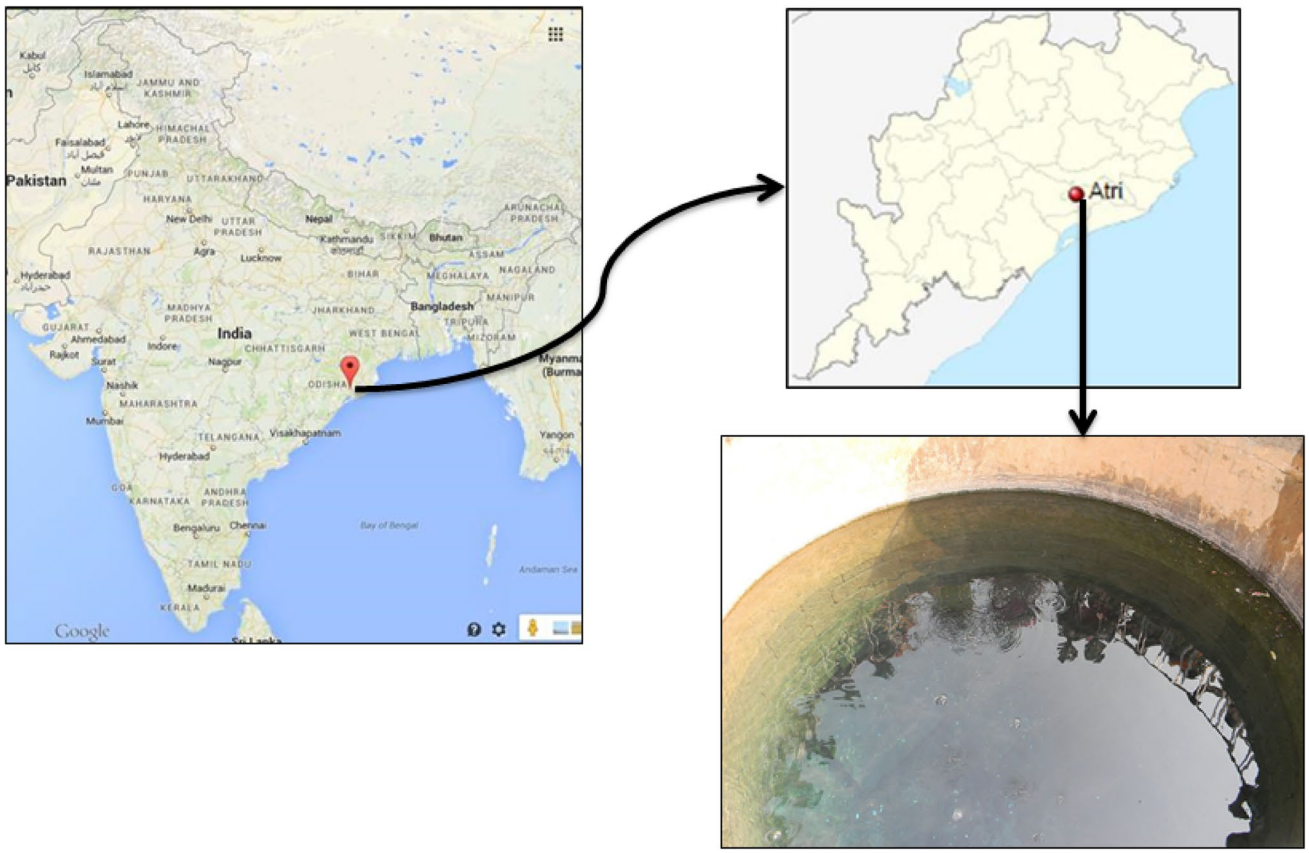


Fig. 1 Geographic location of the alkaline sulfur hot water spring in the Eastern Ghats mobile belt zone

the Gondwana Graben lying at 20°N and 85°E is located in the Eastern Ghats zonal region of India. Temperature of the spring water has been recorded to be 55–58 °C with an alkaline pH of 8.5. These springs are predominantly rich in salts, such as sodium chloride. The analysis of the gaseous samples in these springs has revealed that nitrogen (88–90.5 %) is present in greater concentration compared to dissolved oxygen (1.2–6.6 %). Other components viz., helium, argon, methane, and carbon dioxide has also been detected (Mahala et al. 2012). The sulfurous odor of the spring owe to the presence of dissolved gases, such as hydrogen sulfide.

In the past few decades, the development of next-generation sequencing (NGS) technologies through the construction of metagenomic libraries has provided a better insight into the structural and functional diversity of the microbial communities existing in high-temperature environments (Inskeep et al. 2010; Jimenez et al. 2012; Schoenfeld et al. 2008). Still more recently, the deployment of NGS technology through metatranscriptomic studies has proved to be quite helpful in analyzing the active fraction of the community and their functional response to extreme environmental conditions. Metatranscriptome studies enable researchers to investigate the actively transcribed messenger RNA from a community (Gilbert and Hughes 2011). In addition, mRNA enrichment procedures further helps us in understanding the community's response to survive and adapt to environmental extremes through different mechanisms.

In the present study, we have characterized the metatranscriptome of the microbial communities of an alkaline hot sulfur spring in the Eastern Ghats region applying Illumina-RNA-Seq approach and determined the anaerobic pathways of energy metabolism linked with the aquatic microbiome. We have evaluated in detail the major lineages associated with important gene encoding enzymes of nitrogen, sulfur, and methane metabolism. This study particularly reflects in depth understanding of the survival and adaptation strategy of the microbial members through different processes involved in energy metabolism.

Materials and methods

Sample collection and sampling process

Sediment soil samples were collected from the Attri hot water spring (20°09′N 85°18′E) located within the Eastern Ghats mobile belt region (Fig. 1). The heat flow values

of the thermal spring belt of Attri ranged from 100 to 180 mW/m^2. Owing to this high heating rate, the samples were collected in sterile plastic containers from the hot water spring at a depth of 0–1 m in July, 2013. Temperature and pH were recorded using a Hach pH-meter equipped with a pH and temperature probe. The samples at the collection site were preserved in LifeGuard™ soil preservation solution (Mo-Bio laboratories, USA), immediately frozen in liquid nitrogen and transported to the laboratory for storage at −80 °C. Five replicates of the sediment samples were collected from the same zone for RNA extraction.

Total RNA extraction and mRNA enrichment

Total RNA was isolated from 2 g of sediment soil sample using RNA power soil total RNA isolation kit (MO-BIO Laboratories, Inc., CA) according to manufacturer's instructions. The extracted RNA was dissolved in DEPC treated water and subsequently processed to remove genomic DNA using DNase I (Epicentre Biotechnologies, USA). The absence of DNA was verified using 100 ng of total RNA in a PCR with 16S rRNA gene-specific primers. Subsequently, RNA was quantified by nanodrop spectrophotometer, and its quality was assessed by agarose gel electrophoresis system. Equal quantity of RNA extracted from five samples were pooled together to obtain sufficient quantity of enriched mRNA fraction using MicrobExpress™ mRNA enrichment kit (Ambion, The Netherlands) after removing the 16s and 23s ribosomal RNAs with sequence-based capture probes attached to magnetic beads following manufacturer's protocols.

Construction of cDNA library and Illumina sequencing

The paired-end cDNA library was prepared using Illumina TruSeq RNA sample preparation kit according to manufacturer's instructions. Library preparation was initiated with mRNA fragmentation followed by random priming, first-strand synthesis by reverse transcription, second-strand synthesis (MBI Fermentas, USA), end-repairing, adenylation of 3′ ends, pair-end adapter ligation, and PCR amplification of the paired cDNA ends. The amplified library was analyzed in Bioanalyzer 2100 (Agilent Technologies) using high-sensitivity DNA chip. Following size selection in the range of 400–700 bp, the library was subjected to complete run on MiSeq platform using 2 × 250 bp sequencing kit.

Filtering and assembling of high-quality reads

Low-quality and short (50 bp) reads were removed from the data set using an in-house perl script. Ribosomal RNAs, small RNAs, and transfer RNAs obtained after mapping of the high-quality reads against Rfam and Silva databases were removed using SortMeRNA tool (Kopylova et al. 2012). High-quality mRNA reads were assembled using CLC assembler v4.0 (CLC bio, Cambridge, MD) based on de Bruijn graph algorithm with default parameters (Mismatch cost = 2, Insertion cost = 3, Deletion cost = 3, Length fraction = 0.5, and Similarity fraction = 0.8). The mRNA data set was loaded into the MG-RAST (MetaGenomics-Rapid Annotation using Subsystem Technology) and MEGAN (MEtaGenome ANalyzer) software for structural and functional analysis.

Taxonomic categorization

The mRNA data set was annotated against GenBank (NCBI Non-Redundant), PATRIC, RefSeq, and TrEMBL databases. Taxonomic classification was performed using BLASTX (Altschul et al. 1990) on the MG-RAST v3.0 (Meyer et al. 2008) software with a cutoff *E* value of 1e−5, minimum alignment length of 50 bp, and minimum identity of 60 %. The annotated mRNA data set was further imported to the MEGAN4 (Huson et al. 2011) software for computing abundance of the microbial communities at species level.

Functional categorization

Functional analysis of mRNA data set was performed in MG-RAST module by hierarchical classification with cutoff *E* value of 1e−5, minimum alignment length of 50 amino acids, and minimum identity of 60 % using Clusters of Orthologous Groups (COG) (Tatusov et al. 2001), KEGG (Kyoto Encyclopedia of Genes and Genomes) (Kanehisa and Goto 2000), and SEED (Overbeek et al. 2005) databases. For COG analysis, reads were mapped onto genes with known COG. For KEGG analysis, reads were allotted to different pathways by applying the best hit method. In the case of SEED classification; reads were mapped to the protein sequences with a known functional role.

Data deposition

Metatranscriptome data were deposited in the sequence read archive of National Center for Biotechnology Information under the accession number SRX643298.

Results

Assembly statistics of metatranscriptome data set

A total of 17,501,596 reads were generated from the Illumina sequencing of the metatranscriptome library which

corresponded to 4.53 Gb data (Supplementary Table S1). After the filtration of the low-quality and short reads (50 bp), 7,786,277 number of high-quality reads equivalent to 2.09 Gb data were obtained, while a total of 57,480 mRNA reads were obtained with a mean length of 725 ± 613 bp. Of the mRNA reads that passed quality control, 39,958 (69.5 %) reads contained predicted proteins with a known function and 15,245 (26.5 %) reads contained predicted proteins with unknown function. Remaining reads failed to pass the quality control in MG-RAST pipeline.

Microbial community composition

The community structure and composition in an ecosystem is determined by the physico-chemical properties (Supplementary Table S2). In Attri hot sulfur spring, bacteria (96.8 %) constituted the active fraction of the microbial communities followed by archaea (1.7 %) and eukaryota (1.2 %). The major lineages of the hot spring at different levels showed the dominance of bacterial population (Fig. 2). *Proteobacteria* (28.1 %), *Chloroflexi* (17.8 %), *Cyanobacteria* (9.1 %), *Firmicutes* (8.0 %), *Bacteriodetes* (7.9 %), and *Deinococcus-Thermus* (6.9 %) were the dominant communities. *Deltaproteobacteria* and *Betaproteobacteria* that includes wide variety of aerobic and anaerobic bacteria formed the major *Proteobacteria* classes. *Chloroflexales* (13 %) and *Thermales* (6.4 %) were dominant at order level indicating the abundance of phototrophic and thermophilic bacterial communities in the hot spring. *Chloroflexales* was largely represented by the genus *Roseiflexus* (8.1 %), *Chloroflexus* (4.5 %), and *Oscillochloris* (0.4 %). Speciation of the bacterial population of hot spring communities revealed the presence of many thermophiles viz., *Anaerolinea thermophila*, *Meiothermus ruber, Thermodesulfovibrio yellowstonii*, and *Thermus thermophilus* (Supplementary Table S3). The bacterial diversity of the hot spring constituted both aerobic and anaerobic populations, thereby indicating dual mode of lifestyle of the community members.

Classification of reads associated with energy metabolism

A considerable number of reads were found associated with energy metabolism using different functional identifiers. A total of 28,705 reads were assigned to 23 COG categories, out of which a greater number of reads (2170) were linked with energy production and conversion (Fig. 3). Based on KEGG classification, 15,631 reads were assigned to various functional categories. A total of 988 reads were associated with energy metabolism, out of which 33.7 % of the reads were assigned to nitrogen, sulfur, and methane metabolism. Similarly, under SEED classification, a total of 40,184 reads were mapped to 28 subsystems, out of which 364 and 427 reads were related to nitrogen and sulfur metabolism, respectively. The number of reads linked with anaerobic metabolism indicates that the microbial communities of the Attri hot spring depend on different terminal electron acceptors other than oxygen.

Fig. 2 Phylogenetic assignment of the metatranscriptomic reads based on predicted proteins using BLASTX against different databases available in MG-RAST. Each *slice* indicates the percentage of reads with predicted proteins annotated to the indicated taxonomic level. The unclassified reads were represented by the *gray* fraction

Microorganisms utilize different terminal electron acceptors (nitrate, sulfate, elemental sulfur, and carbon dioxide) other than oxygen to survive and adapt to the high-temperature environment. The involvement of different genera in the anaerobic pathways indicates the diverse functional role of the hot spring microbial communities (Fig. 4). To further understand the survival of the microorganisms in the high-temperature environment through anaerobic pathways, we aim here to study the different gene encoding enzymes of nitrogen, sulfur, and methane metabolism, and the various taxa involved in these processes.

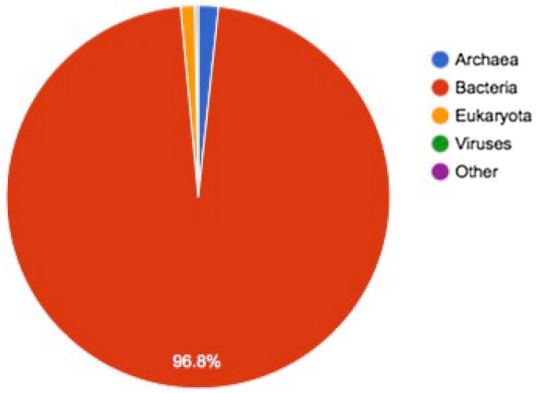
Nitrogen metabolism

The anaerobic lifestyle of the hot spring communities was verified through the involvement of substantial number of enzymes in nitrogen metabolism. Nitrate acts as the terminal electron acceptor during anaerobic respiration in microbes. Nitrate is formed during nitrification by the oxidation of ammonia to nitrite followed by the oxidation of nitrite to nitrate. Hydroxylamine is the intermediate compound formed during the oxidation of ammonia to nitrite. In Attri hot spring, the conversion of hydroxylamine to nitrite was performed by *Anaeromyxobacter* in the presence of the enzyme hydroxylamine oxidase. Nitrite formed was then oxidized to produce nitrate. Similarly, nitrate reduction process was carried out by a set of denitrifying genes involved in the denitrification reaction. Genes encoding nitrate and nitrite ammonification was enrichingly observed in *Proteobacteria* and *Verrumicrobia*. Nitrate was reduced to nitrite by *Opitutus terrae* and *Cronobacter turicensis*, and this process was catalyzed by nitrate reductase. Again, nitrite was reduced to ammonia by *Candidatus Accumulibacter phosphatis* in the presence of the enzyme nitrite reductase. Different gene encoding enzymes of nitrogen metabolism were associated with various microbial phylotypes of the hot sulfur spring (Table 1).

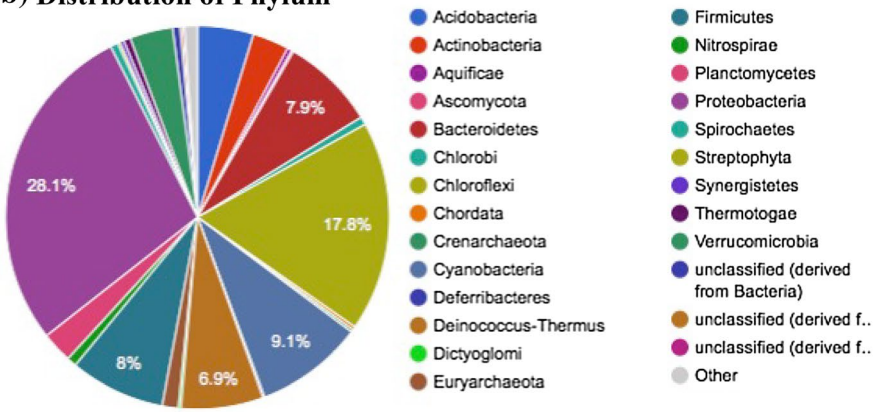
Sulfur metabolism

Microorganisms depend on sulfate and elemental sulfur as the terminal electron acceptor during anaerobic metabolism.

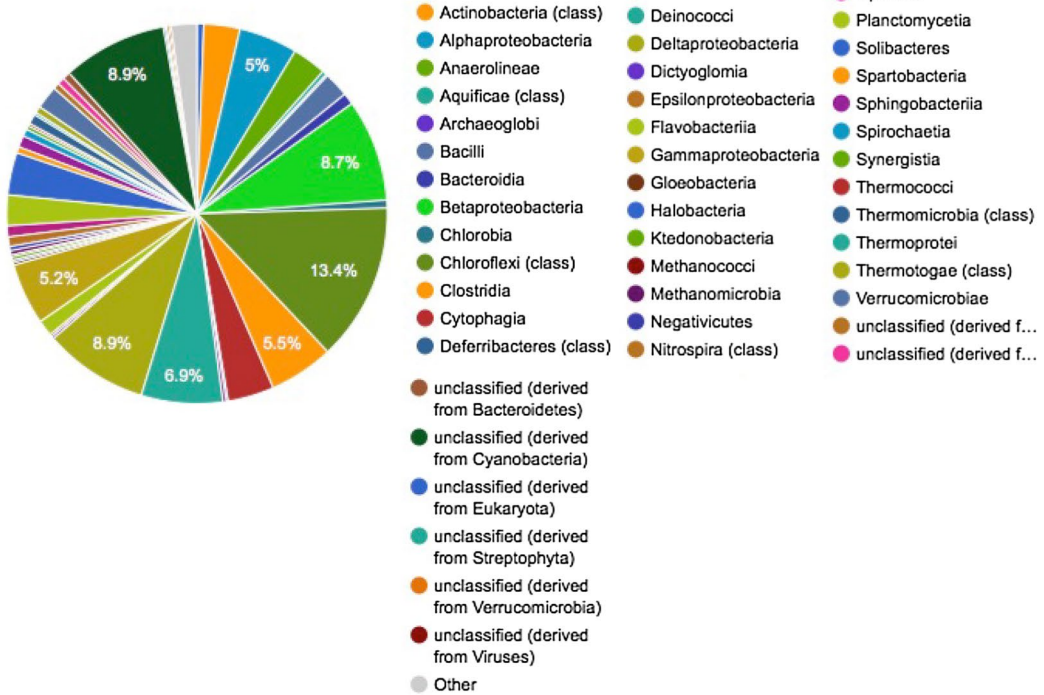
(a) Distribution of Domain



(b) Distribution of Phylum



(c) Distribution of Class



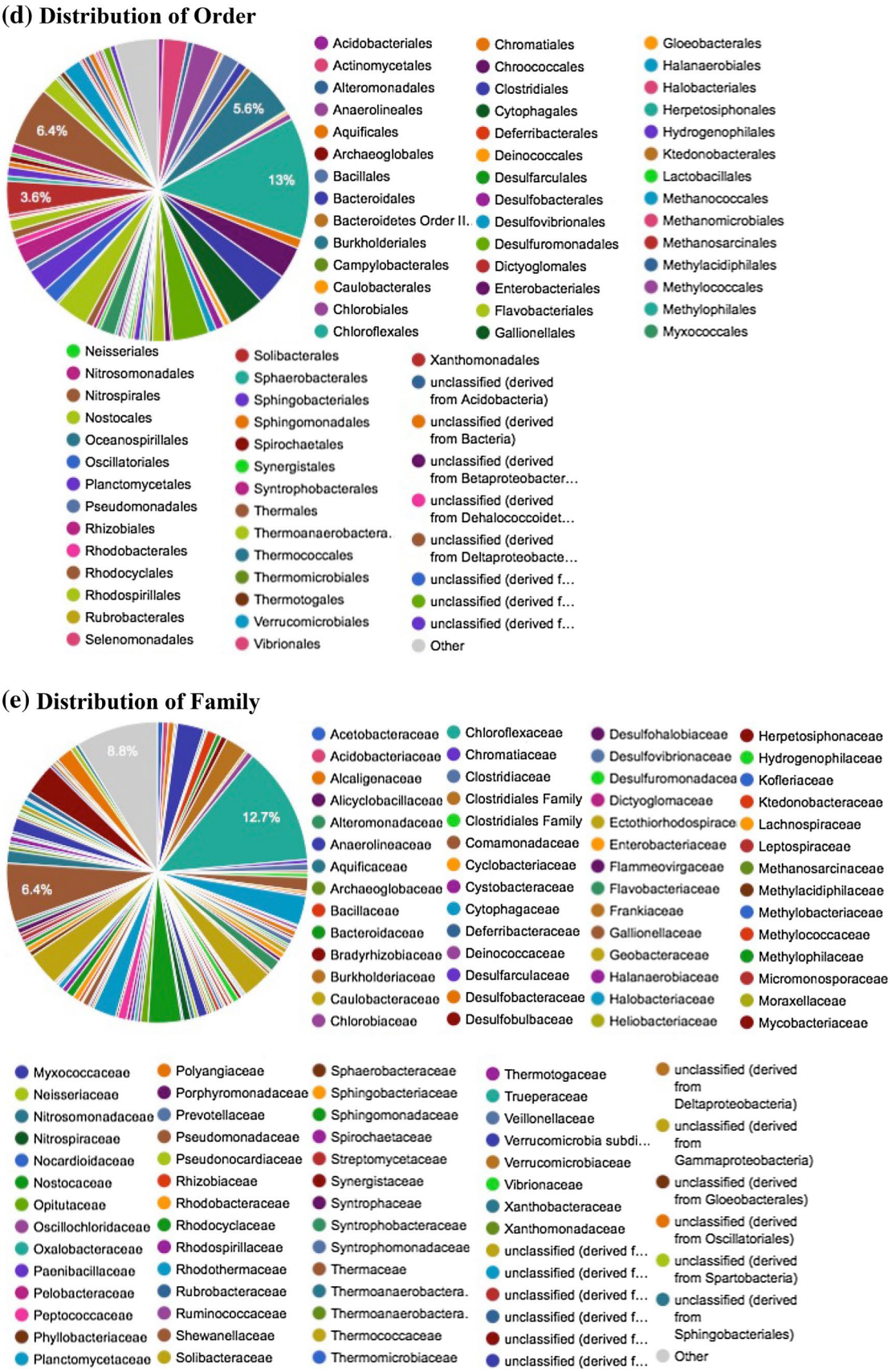


Fig. 2 continued

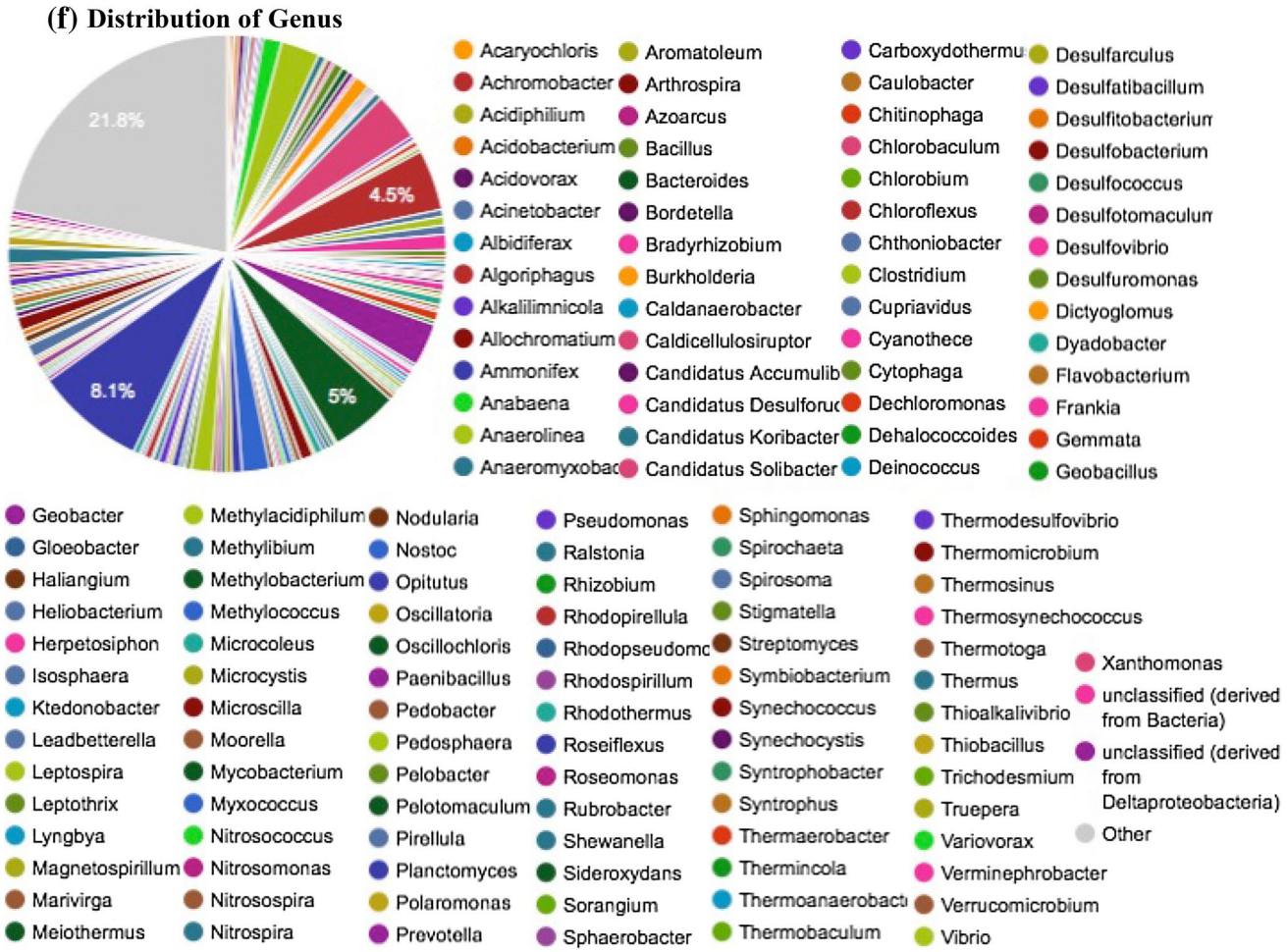
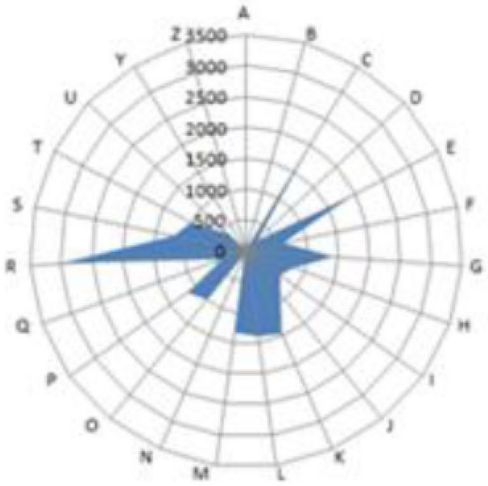


Fig. 2 continued

The phototrophic communities of the hot spring (*Cyanothece, Salinibacter*) acted upon 3′-phosphoadenosine-5′-phosphosulfate (PAPS) to produce adenosine-5′-phosphosulfate (APS) in the presence of the enzyme 5′-bisphosphate nucleotidase, which in turn yielded sulfate with the release of ATP. Likewise, sulfate reduction was activated by the formation of APS and PAPS. APS was formed from sulfate by *Thiobacillus denitrificans* in the presence of the enzyme sulfate adenylyltransferase, associated with inorganic sulfur assimilation. APS and PAPS are intermediates in the reduction of sulfate to sulfite, an exothermic reaction that is carried out by sulfate-reducing bacteria. PAPS compound was further reduced by *Pirellula staleyi* and *Thermosynechococcus elongatus* to produce sulfite through the action of phosphoadenosine phosphosulfate reductase. Sulfite thus formed was acted upon by sulfite reductase to produce hydrogen sulfide gas through a process called dissimilatory sulfate reduction. The gene encoding enzymes of sulfur metabolism suggest the involvement of diversified communities of the hot spring in this process (Table 2).

Methane metabolism

Methanogenesis is a form of anaerobic respiration in microbes, where the terminal electron acceptor is carbon. Depending upon pH and temperature, microorganisms utilize carbon from different organic compounds in the ecosystems. In Attri hot sulfur spring, formate was oxidized by the phototrophic bacteria (*Chloroflexus* and *Roseiflexus*) to yield carbon dioxide in the presence of the enzyme formate dehydrogenase. Carbon dioxide thus formed was reduced to carbon monoxide and used in the formation of acetyl-CoA in the presence of the enzymes carbon-monoxide dehydrogenase and acetyl-CoA synthase. Acetyl-CoA was also produced from acetate by the action of enzyme acetyl-CoA synthetase. Acetyl-CoA is utilized by thermophilic archaea (*Archaeoglobus*) to fix carbon dioxide that act as the carbon source required for growth of these organisms and used as an electron acceptor for methane production. Different gene encoding enzymes of methane metabolic process were observed among the diversified microbial members of the hot spring (Table 3).



A	RNA processing and modification
B	Chromatin structure and dynamics
C	Energy production and conversion
D	Cell cycle control cell division chromosome partitioning
E	Amino acid transport and metabolism
F	Nucleotide transport and metabolism
G	Carbohydrate transport and metabolism
H	Coenzyme transport and metabolism
I	Lipid transport and metabolism
J	Transcription
K	Translation, ribosomal structure and biogenesis
L	Replication recombination and repair
M	Cell wall/membrane/envelope biogenesis
N	Cell motility
O	Posttranslational modification protein turnover chaperones
P	Inorganic ion transport and metabolism
Q	Secondary metabolites biosynthesis transport and catabolism
R	General function prediction only
S	Function unknown
T	Signal transduction mechanisms
U	Intracellular trafficking secretion and vesicular transport
Y	Nuclear structure
Z	Cytoskeleton

Fig. 3 Functional assignment of metatranscriptome reads using BLASTX against COG database. The *number* indicates the total reads affiliated to the predominant COG identifiers. Maximum reads were allotted to specific COG identifiers viz., C, E, G, K, L, M, O, P, and R

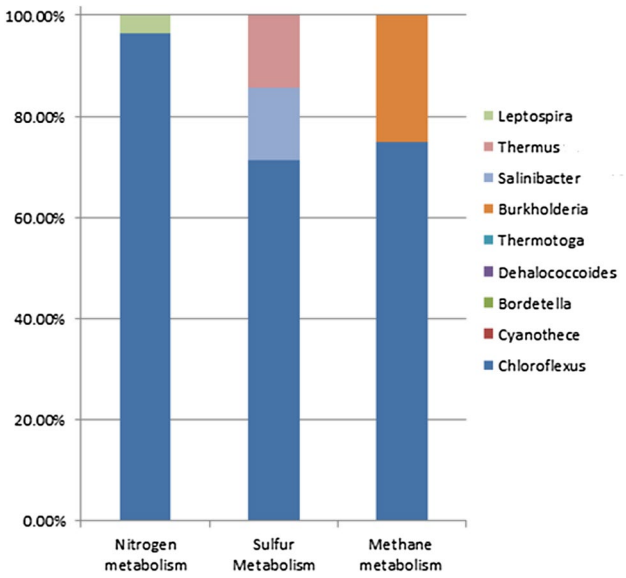


Fig. 4 Taxonomic classification of metatranscriptomic reads involved in energy metabolism (KEGG identifiers) specifically related to nitrogen, sulfur, and methane metabolism at genus level. The taxonomic assignment was performed by BLASTX against NCBI-nr database and evaluated using the MEGAN v4.0 software, which provides phylogenetic classification at various levels, based on the reads

Discussion

The present study was focused on the survival and adaptation of the microbial communities of hot sulfur spring through the involvement of functional gene encoding enzymes in anaerobic metabolic pathways which were determined by the construction and sequencing of mRNA-enriched metatranscriptome library using Illumina MiSeq approach. Metatranscriptomic studies have traditionally involved the use of either microarrays or mRNA derived cDNA clone libraries (expressed sequence tags (EST) libraries) (Poretsky et al. 2005; Parro et al. 2007; Gilbert et al. 2008). However, more recently, life in extreme environments has been decoded through several studies based on different meta-omics approach through the deployment of NGS technologies (Urich et al. 2008; Liu et al. 2011; Jimenez et al. 2012; Krohn-Molt et al. 2013; Inskeep et al. 2013; Wemheuer et al. 2013). The functional aspect of the microbial communities in the natural environment is better elucidated by analyzing the mRNA profiles; hence, here, we applied the metatranscriptomic approach to investigate the functional gene encoding enzymes associated with the anaerobic metabolic processes of the hot spring microbiomes. Phylogenetic and functional assignments were obtained by directly aligning the mRNA reads to the complete NCBI prokaryote genome database that includes draft genomes (Leimena et al. 2013).

Phylogenetic classification of Attri hot sulfur spring microbiomes showed that the microbial communities were dominated by bacterial domains that was quite similar to the diversity reported in other sulfur thermal springs (Skirnisdottir et al. 2000; Stout et al. 2009). The bacterial communities found in the sediment sample of Attri

Table 1 Sequences associated with specific enzyme and taxa within nitrogen metabolism using KEGG pathways

Number of sequences assigned	EC number	KEGG orthology	Enzyme name	Organism	Phylum	*E* value
1	EC:1.7.99.4	KO0372	Nitrate reductase (*nar*)	*Opitutus terrae* *Cronobacter turicensis*	Verrucomicrobia Proteobacteria	3e−32 1e−68
1	EC:1.7.7.1	KO0366	Nitrite reductase (*nirA*)	*Chloroflexus*	Chloroflexi	0
1	EC:1.7.1.4	KO0362	Nitrite reductase (*nirB*)	*Burkholderia pseudomallei*	Proteobacteria	e−109
1	EC:1.7.3.4	K10535	Hydroxylamine oxidase (*hao*)	*Anaeromyxobacter*	Proteobacteria	8e−38
1	EC:1.7.7.2	KO0367	Ferredoxin-nitrate reductase (*narB*)	*Nostoc*	Cyanobacteria	5e−48
1	EC:1.7.1.4	KO0363	Nitrite reductase (*nirD*)	*Candidatus Accumulibacter phosphatis*	Proteobacteria	2e−31
1	EC:1.7.2.1	KO0368	Nitrite reductase (NO-forming)	*Cupriavidus necator*	Proteobacteria	4e−15
1	EC:1.7.1.1	KO0360	Nitrate reductase (NADH)	*Saccharopolyspora erythraea*	Actinobacteria	6e−07

Table 2 Sequences associated with specific enzyme and taxa within sulfur metabolism using KEGG pathways

Number of sequences assigned	EC number	KEGG orthology	Enzyme name	Organism	Phylum	*E* value
1	EC:1.8.4.8	K00390	Phosphoadenosinephosphosulfate reductase (*cysH*)	*Thermosynechococcus elongatus*	Cyanobacteria	9e−93
1	EC:2.7.7.4	K00958	Sulfate adenylyltransferase (*met3*)	*Thiobacillus denitrificans*	Firmicutes	0
1	EC:1.8.1.2	K00381	Sulfite reductase (NADPH) Hemoprotein beta-component (*cysI*)	*Burkholderia pseudomallei*	Proteobacteria	1e−48
1	EC:3.1.3.7	K01082	5′-Bisphosphate nucleotidase (*cysQ*)	*Salinibacter ruber*	Chlorobi	2.80e−018
1	EC:2.7.7.4	KO0957	Sulfate adenylyltransferase (*cysD*)	*Opitutus terrae*	Verrucomicrobia	e−148
1	EC:2.7.7.4	KO0956	Sulfate adenylyltransferase subunit 1	*Acidothermus cellulolyticus*	Actinobacteria	2e−54
1	EC:1.8.7.1	KO0392	Sulfite reductase (ferredoxin)	*Nostoc punctiforme*	Cyanobacteria	e−157

alkaline hot water spring were different from the microbial population detected in the soil samples and mangrove sediments of acidic hot water spring of Colombian Andes (Jimenez et al. 2012) which suggests considerable variation exists among the microbial types of both the hot springs. Comparatively lesser diversification was observed in the communities of Attri hot spring consisting of archaea and eukaryota. Archaea may use more energy in maintaining metabolism in extreme environments and are thus less able to diversify (Stout et al. 2009), while the growth of eukaryotic species was probably limited due to elevated temperature.

Microorganisms in extreme environments to drive physiologically important processes depend on different energy metabolic pathways that includes both aerobic and anaerobic metabolism. Anaerobic metabolism is particularly significant in habitats, where terminal electron acceptors other than oxygen become important, such as nitrate, fumarate, arsenate, selenate, thiosulfate, elemental sulfur, sulfate, oxidized metal ions, and carbon dioxide. The anaerobic metabolic processes viz. nitrogen, sulfur, and methane metabolism are found to occur in diversified group of microorganisms. *Proteobacteria*, the major bacterial phylum identified in many thermal ecosystems (Elshahed

Table 3 Sequences associated with specific enzyme and taxa within methane metabolism using KEGG pathways

Number of sequences assigned	EC number	KEGG Orthology	Enzyme name	Organism	Phylum	*E* value
2	EC:1.2.1.2	K00123	Formate dehydrogenase alpha subunit	*Chloroflexus* *Burkholderia pseudomallei*	Chloroflexi Proteobacteria	0 2.00e−003
1	EC:6.2.1.1	–	Acetyl-CoA synthetase (*acs*)	*Chloroflexus*	Chloroflexi	0
1	EC:2.3.1.169	K14138	Carbon-monoxide dehydrogenase/acetyl-CoA synthase subunit alpha	*Thermodesulfovibrio yellowstonii*	Nitrospirae	6e−56
1	EC:2.3.1.169	KO0190	Carbon-monoxide dehydrogenase/acetyl-CoA synthase subunit beta	*Carboxydothermus hydrogenoformans*	Firmicutes	e−107
1	EC:1.2.99.2C	K00198	Carbon-monoxide dehydrogenase catalytic subunit (*cooS*)	*Thermoanaerobacter tengcongensis*	Firmicutes	2e−81
1	EC:1.2.99.2F	K00196	Carbon-monoxide dehydrogenase iron sulfur subunit (*cooF*)	*Archaeoglobus fulgidus*	Euryarchaeota	4e−08
1	EC:1.2.99.2L	K03520	Carbon-monoxide dehydrogenase large subunit (*cutL*, *coxL*)	*Nocardioides*	Actinomycetes	4e−25
1	EC:1.2.99.2 M	K03519	Carbon-monoxide dehydrogenase medium subunit (*cutM*, *coxM*)	*Petrotoga mobilis*	Thermotogae	1e−12
1	EC:1.2.99.2S	K03518	Carbon-monoxide dehydrogenase small subunit (*coxS*)	*Chloroflexus aggregans*	Chloroflexi	5e−84

et al. 2003; Yamamoto and Takai 2011; Jimenez et al. 2012) was associated with nitrate and nitrite ammonification. In addition, gene encoding enzymes of nitrogen metabolism were observed among different phylotypes of the hot sulfur spring. This was due to the fact that nitrification and denitrification ability of a variety of phylogenetically unrelated organisms is widespread and this has been presumably acquired through horizontal gene transfer (Braker and Tiedje 2003).

Furthermore, the phototrophs belonging to the phylum *Chlorobi* and *Cyanobacteria* were involved in sulfur metabolic processes which indicated a better adaptation of these organisms to alkaline waters as compared to the acidic waters (Elshahed et al. 2003). The anaerobic green sulfur photoautotrophs were represented by *Chlorobi* (Beatty et al. 2005), while the occurrence of *Cyanobacteria* indicated a better survival ability in a wide variety of habitats. Besides, the sulfate reduction process was carried out by the members of *Firmicutes* that are able to survive in different environments due to metabolic and physiological versatility (Pandey et al. 2013). Similarity was observed in the involvement of diversified community members in sulfur metabolism in the sulfur-rich springs of Attri and Zodletone (Elshahed et al. 2003).

Likewise, the members of the phylum *Chloroflexi*, (filamentous anoxygenic phototrophs) mostly *Roseiflexus* and *Chloroflexus* spp. (van der Meer et al. 2010; Ward et al. 2012) were noticed as the dominant green non-sulfur bacteria in the hot spring associated with the methane metabolic reactions involving the enzyme formate dehydrogenase that leads to the formation of carbon dioxide, used as a terminal electron acceptor by the microorganisms (Ferry, 1990). The abundance of chlorophototrophic *Chloroflexi* is reflective of their previously established physiological diversity, including photoheterotrophy, photoautotrophy, photomixotrophy, and oxic and anoxic chemoorganotrophy (Hanada et al. 2002; van der Meer et al. 2010; Zarzycki and Fuchs 2011). Furthermore, the thermophilic organisms were involved in fixing carbon dioxide required for methane production. Temperature is the main factor that shapes microbial communities in the hot springs with different locations (Miller et al. 2009). Notable similarity was observed in the involvement of different community members in methane metabolism in Attri as well as in Malaysian hot springs (Chan et al. 2015).

This study improves our understanding of various gene encoding enzymes of energy metabolism and suggests their importance in the survival of hot spring microbial

population. Even though, a brief idea regarding the diverse mode of adaptation of the hot spring microbial communities was generated from this study, still additional sequence coverage is essential to establish more complete assemblies for the identification of poorly characterized and unclassified sequences.

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Conflict of interest The authors declare no conflict of interest.

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