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Functional metagenomics and microbial community structure analysis of Indian hot springs

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Abstract

Hot springs are the high temperature environments that serve as the ideal habitats for a number of thermophilic microorganisms. Exploration of microbial communities of these environments has both basic and applied aspects. The limit of the traditional culture based methods to access microbial world has now been extended many folds by the culture-independent approach of metagenomics. World over metagenomic studies of hot springs have resulted in the identification of several novel prokaryotic life forms as well gene(s) of immense industrial applications. There are several thermal springs located within the territorial boundary of India and quite a few of them have been explored for metagenomic investigations as well. This review describes community structure elucidation as well as functional gene identification in the community genome of some hot springs in India.

Key words: Indian hot springs; metagenomics; community structure; functional genes

Introduction

Thermal springs are hot aquatic ecosystems that are widely distributed throughout the world. They are most numerous in volcanically active areas (Tanti and Saha, 1993) and have great geological and environmental relevance. In the past few decades these high temperature environments have also been proved to be the ideal habitats of thermophilic and hyperthermophilic microbes (Chaudhuri *et al.*, 2017) that have immense industrial applications. The commercial uses of microbial thermostable metabolites such as enzymes, have made hot springs attractive sites to explore the associated microbial wealth. Over the years, several novel microorganisms have been isolated and identified and their commercial applications have been established by employing the traditional culture based methods. Culture based methods however, give access to a very small proportion of the microbial population compared to the overall size of the microbial community in an environment (Staley and Konopka, 1985). It may be due to various reasons such as lack of proper media formulation, inability to provide in-vitro conditions similar to the environmental conditions etc. (Handelsman, 2004). In the recent time, the culture independent approach of metagenomics and associated technologies have revolutionized the study of microbial diversity, adaptation and evolution (Riesenfeld *et al.,* 2004). It has also become a modern tool for functional characterization of microbial life forms. Worldover, many hot springs have been explored for estimation of the associated microbial diversity as well as to hunt the novel gene(s) encoding important metabolites by employing the techniques of metagenomics (López-López *et al.,* 2013). The approach of metagenomics is fastly replacing the traditional culture based approach to study the phylogenetics as well as functional attributes of microbial diversity in Indian hot springs as well. This review describes the outcome of the metagenomic studies undertaken in Indian hot springs.

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Functional metagenomics in Indian hot springs

The functional metagenomics is as an efficient method consisting of extraction, cloning and analysis of the entire genetic component of a habitat, thus allowing the investigation of wide diversity of individual genes and their products

(Rondon *et al.,* 2000). Till date various reports have been published on functional metagenomic studies of different habitats nationally and internationally (Saxena *et al.,* 2017; Schmeisser *et al.,* 2017). However, there are only handful of reports on function based metagenomic studies carried out in Indian hot springs. The literature survey revealed that hot springs in India, that are explored for the isolation of some enzymatic and other genes are Manikaran hot spring (Himachal Pradesh), a geothermal spring from North Himalayan region (Jammu & Kashmir), Chumthang hot spring (Ladakh), Puga hot spring (Ladakh), Taptapani hot spring (Odisha) and Tapovan Hot spring (Uttarakhand). Some novel thermostable enzymatic genes have been isolated from the Indian hot spring metagenome by Gupta *et al.,* (2012) and Singh *et al.,* (2015). The former group in their study isolated a novel β-galactosidase enzyme of 447 amino acid residues belonging to glycoside hydrolase family, from a geothermal spring in North Himalayan region. The temperature and pH optima of this enzyme were found to be 65°C and 8.0, respectively. It was showing 80% of the enzymatic activity even at pH 10.0. A thermophilic pectinase was isolated from Manikaran hot spring that was composed of an ORF of 1311bp (Singh *et al.,* 2012). This enzyme was found to have temperature and pH optima of 70°C and 7.0, respectively, and active over a broad temperature and pH range. The Chumthang hot spring (Ladakh) was also screened for protease enzyme where after screening of 9000 clones, a single clone showing protease activity was identified. Sequence analysis of the protease gene revealed this to be a 363 amino acids long protein (Singh *et al.,* 2015). One thermo-alkali stable and surfactant stable endogluconase gene consisting of 554 amino acids was identified from metagenomic library of Puga hot spring, Ladakh using functional screening (Gupta *et al.,* 2017). The enzyme exhibited activity over a broad range of pH and temperature with optima at pH 8.0 and 65°C, respectively. Lipase enzymes were obtained by cloning and analysing the metagenome of Taptapani hot spring (Odisha) by Sahoo *et al.* (2017). The lipase enzyme was stable over a range of pH 7.0-9.0 and temperature of 55-75°C, and hydrolyzed a wide range of esters. Rawat and Joshi, (2015) have identified two amylase positive clones in a metagenomic library created from a hot spring located in Tapovan region of Uttarakhand.

Elucidation of microbial diversity and community structure in Indian hot springs

For the study of bacterial diversity in any environment, 16S rRNA gene analysis plays a key role, both in cultural as well as non cultural approaches. The rRNA sequences are popular in bacteriology because it is often easier to identify bacteria by specific nucleic acid sequences rather than by their biochemical or physiological traits (Marchesi *et al.,* 2001). The partial 16S rRNA based metagenomic approach has been utilized globally for the study of resident microbiota in extreme environments (Chan *et al.,* 2015). The first microbiological investigation on any hot spring using metagenomic approach in India was done by Ghosh *et al.,* (2003). They studied the bacterial diversity of Bakreshwar hot spring, West Bengal by preparing 16S rRNA clonal library and concluded that there was abundance of Proteobacteria followed by Cynobacteria and green non sulphur bacteria. Sharma *et al.,* (2015) performed metagenomic study to determine microbial diversity of Soldhar hot spring in Uttarakhand using clonal library and denaturing gradient gel electrophoresis (DGGE). They also detected Proteobacteria as the most predominant group. Similar observations with regard to the predominance of Proteobacteria in hot spring were recorded by Mohanrao *et al.,* (2016) while analysing the microbial diversity of Tattapani hot spring, Himachal Pradesh.With the use of advance sequencing techniques such as high throughput sequencing or Next generation sequencing (NGS), there is a boom in the reports of microbial diversity analysis of different environments including hot springs. Presently, a number of hot springs have been investigated for whole microbial community analysis using these advance techniques. The high throughput sequencing approach is used first by amplifying the conserved regions of 16S rRNA gene followed by sequencing on different platforms like FLX amplicon pyrosequencing (Ghelani *et al.,* 2015); Ion Torrent PGM platform (Mangrola *et al.,* 2015); Illumina sequencing (Panda *et al.,* 2016). Many hot springs in India, like Atri and Taptapani (Sahoo *et al.,* 2015), Tulsi Shyam (Ghelani *et al.,*

2015), Lasundra (Mangrola *et al.,* 2015), Manikaran hot spring (Bhatia *et al.,* 2015), Jakrem hot spring (Panda *et al.,* 2015), Tuwa hot spring (Mangrola *at al.,* 2015), Deulajhari hot spring (Singh *et al.,* 2016), Unkeshwar hot spring (Mehetre *et al.,* 2016), Yumthang hot spring (Panda *et al.,* 2016) and Soldhar hot spring (Sharma *et al.,*

Table 1. Microbial community structure in Indian hot springs

S.N.	Hot spring	Metagenomic approach	Microorganisms present (%)	References
1.	Bakreshwar, West Bengal	16S rRNA gene cloning	Proteobacteria (40%), Cyanobacteria (32%), Green nonsulfur bacteria (12%), Low- GC gram positive bacteria (16%)	Ghosh *et al.,* 2003
	Two hot springs at Bakreshwar	High throughput sequencing, illumina Miseq platform	One was dominated by Fermicutes (65.85%) followed by phylum Synergistetes (27.24%). Another was also dominated by Fermicutes (96.10%)	Chaudhuri *et al.,* 2017
2.	Soldhar, Uttarakhand	a)16S rRNA cloning, DGGE, functional nif gene cloning	Proteobacteria (50%), Aquificae (38%), Deinococcus-Thermus (9%)	Sharma *et al.,* 2015
		b) High throughput sequencing in Ion Torrent sequencing platform	Proteobacteria (88.8%), Deinococcus-Thermus (7.5%), Actinobacteria (2.3%), Fermicutes (1.07%), were found in predominance whereas Bacteroidetes, Verrucomicrobia, Aquificae and Acidobacteria were found in lower abundance.	Sharma *et al.,* 2017
3.	Atri and Taptapani, Odisha	Deep sequencing analysis using illumine bar coded platform	Proteobacteria (45.17%) dominated the Taptapani followed by Bacteriodetes (23.43%) and Cyanobacteria (10.48%) while in the Atri sample Chloroflexi (52.39%), Nitrospirae (10.93%) and Proteobacteria (9.98%) were dominating.	Sahoo *et al.,* 2015
4.	Tulsi Shyam, Gujarat	High throughput sequencing on 454 GS FLX pyrosequencing platform	98.2% of metagenome was of bacterial origin, 1.5% eukaryotic, 0.3% unidentified. Abundant bacterial phyla were Firmicutes (65.38%), Proteobacteria (21.21%) and unclassified bacteria (10.69%).	Ghelani *et al.,* 2015
5.	Lasundra, Gujarat	High throughput sequencing using Ion Torrent PGM platform	99.21% sequences were of bacterial origin, 0.43% eukaryotic and 0.11% belonged to archea. Abundant	Mangrola *et al.,* 2015

			prokaryotic phyla were Firmicutes (95.5%) and Proteobacteria (2%),	
6.	Manikaran, Himachal Pradesh	High throughput sequencing using Illumina Miseq platform	Dominant phyla were Firmicutes (28-84%), Aquificae (2-64%), Deinococcus-Thermus (1-18%). Crenarchaeota (0.04-3%) was the main archeal phylum.	Bhatia *et al.*, 2015
7.	Jakrem, Meghalaya	High throughput sequencing using illumina sequencing platform	Dominated bacterial phyla were Firmicutes (61.60%), Chloroflexi (21.96%) and unclassified bacteria (1.2%).	Panda *et al.*, 2015
8.	Tuwa hot spring, Gujarat	Shotgun sequencing, Ion-Torrent PGM platform	99.1% sequences belonged to bacteria, 0.3% to eukaryotic, 0.2% virus and 0.05% from archea. Firmicutes (97%), Proteobacteria (1.3%) and Actinobacteria (0.4%) were the dominating phyla reported.	Mangrola *et al.*, 2015
9.	Deulajhari, Odisha	High throughput illumina sequencing platform	Major phyla were Chloroflexi (22.98%), Proteobacteria (15.51%), Acidobacteria (14.51%), Chlorobi (9.52%), Nitrospirae (8.54%) and Armatimonadetes (7.07%).	Singh *et al.*, 2016
10.	Unkeshwar, Maharashtra	Shotgun sequencing, Illumina Hi seq 2500	99.98% sequences belonged to bacteria, 0.01% archea and 0.01% viruses. The dominant phyla found were Actinobacteria (56%), Verrucomicrobia (24%), Bacteriodetes (13%), Deinococcus-Thermus (3%) and Firmicutes (2%)	Mehetre *et al.*, 2016
11.	Yumthang, Sikkim	Illumina Mi-seq technology	Dominant phyla were Proteobacteria (83.68%), Bacteroidetes (10.93%) and Thermi (1.78%).	Panda *et al.*, 2016
12.	Hot spring from North Himalayan region	High throughput sequencing for exploring bacterial diversity by Laccases gene: the multi copper enzymes	Bacteroidetes (74.28%), Proteobacteria (24.53%), Cellvibrio (6.14%)	Gupta *et al.*, 2017

2017) have been studied for whole microbial community analysis using the above advanced techniques (Table 1). These studies indicated an abundance of bacterial phyla over archea and viruses. In most of the Indian hot springs bacterial community comprised primarily of the members of the phylum Proteobacteria, Fermicutes, Bacteriodetes, Dienococcus-Thermus, Aquificae and Actinobacteria with varied level of abundance. For instance, the percentage of Proteobacterial

population in different hot springs varied from 1.3% to 88% (Mangrola *et al.*, 2015; Sharma *et al.*, 2017). Similarly, the presence of phylum Firmicutes varied from 2% to 97% in different hot springs (Mangrola *et al.*, 2015; Mehetre *et al.*, 2016). On the other hand, some phyla like Chloroflexi, Synergistetes, Nitrospirae, Crenarchaeota were detected in just a few hot springs only (Bhatia *et al.*, 2015; Sahoo *et al.*, 2015; Chaudhuri *et al.*, 2017). The variation in the relative abundance of microbial types in these hot springs may be due to their different physicochemical conditions. At genus level, *Bacillus*, *Geobacillus*, *Paenibacillus*, *Clostridium*, *Pseudomonas* and *Meiothermus* were found in abundance in Indian hot springs studied so far (Panda *et al.*, 2016; Sharma *et al.*, 2015; Gupta *et al.*, 2017). Apart from these known microbial phyla or genera, the NGS of hot springs in Indian also resulted in identification of many unclassified sequences that remained taxonomically unresolved (Panda *et al.*, 2016; Sharma *et al.*, 2017). This indicates the possibility of the presence of potentially novel microbes in these hot springs.

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References

Bhatia, S., Batra, N., Pathak, A., Green, S.J., Joshi, A. and Chauhan, A. 2015. Metagenomic evaluation of bacterial and archaeal diversity in the geothermal hot springs of Manikaran, India**. *Genome Announcements*, *3*(1):e01544-14.**

Chan, C.S., Chan, K.G., Tay, Y.L., Chua, Y.H. and Goh, K.M. 2015. Diversity of thermophiles in a Malaysian hot spring determined using 16S rRNA and shotgun metagenome sequencing. ***Frontiers in Microbiology,*** *6*:177.

Chaudhuri, B., Chowdhury, T. and Chattopadhyay, B. 2017. Comparative analysis of microbial diversity in two hot springs of Bakreshwar, West Bengal, India. ***Genomics*** *Data*, *12*:122-129.

Ghelani, A., Patel, R., Mangrola, A. and Dudhagara, P. 2015. Cultivation-independent comprehensive survey of bacterial diversity in Tulsi Shyam hot springs, India. ***Genomics*** *Data*, *4*:54-56.

Ghosh, D., Bal, B., Kashyap, V.K. and Pal, S. 2003. Molecular phylogenetic exploration of bacterial diversity in a Bakreshwar (India) hot spring and culture of Shewanella-related thermophiles. ***Applied and Environmental Microbiology,*** *69*(7):4332-4336.

Gupta, P., Mishra, A.K. and Vakhlu, J. 2017. Cloning and characterization of thermo-alkalistable and surfactant stable endoglucanase from Puga hot spring metagenome of Ladakh (j&k). ***International Journal of Biological Macromolecules,*** *103*:870-877.

Gupta, R., Govil, T., Capalash, N. and Sharma, P. 2012. Characterization of a glycoside hydrolase family 1 β-galactosidase from hot spring metagenome with transglycosylation activity. ***Applied Biochemistry and Biotechnology,*** *168*(6):1681-1693.

Gupta, V., Gupta, N., Capalash, N. and Sharma, P. 2017. Bio-prospecting bacterial diversity of hot springs in northern Himalayan region of India for laccases. ***Indian Journal of Microbiology,*** *57*(3):285-291.

Handelsman, J. 2004. Metagenomics: application of genomics to uncultured microorganisms. ***Microbiology and Molecular Biology Reviews,*** *68*(4):669-685.

López-López, O., Cerdán, M.E. and González-Siso, M.I. 2013. Hot spring metagenomics. ***Life,*** *3*(2):308-320.

Mangrola, A., Dudhagara, P., Koringa, P., Joshi, C.G., Parmar, M. and Patel, R. 2015. Deciphering the microbiota of Tuwa hot spring, India using shotgun metagenomic sequencing approach. ***Genomics Data,*** *4*:153-155.

Mangrola, A.V., Dudhagara, P., Koringa, P., Joshi, C.G. and Patel, R.K. 2015. Shotgun metagenomic sequencing based microbial diversity assessment of Lasundra hot spring, India. ***Genomics data***, *4*:73-75.

Marchesi, J.R., Weightman, A.J., Cragg, B.A., Parkes, R.J. and Fry, J.C. 2001. Methanogen and bacterial diversity and distribution in deep gas hydrate sediments from the Cascadia Margin as revealed by 16S rRNA molecular analysis. *FEMS* ***Microbiology Ecology,*** *34*(3):221-228.

Mehetre, G.T., Paranjpe, A.S., Dastager, S.G. and Dharne, M.S. 2016. Complete metagenome sequencing based bacterial diversity and functional insights from basaltic hot spring of Unkeshwar, Maharashtra, India. ***Genomics Data,*** *7*:140-143.

Mohanrao, M.M., Singh, D.P., Kanika, K., Goyal, E. and Singh, A.K. 2016. Deciphering the microbial diversity of Tattapani hot water spring using metagenomic approach. ***International Journal of Agricultural Sciences and Research,*** *6*:371-382.

Panda, A.K., Bisht, S.S., Kumar, N.S. and De Mandal, S. 2015. Investigations on microbial diversity of Jakrem hot spring, Meghalaya, India using cultivation-independent approach. ***Genomics Data,*** *4*:156-157.

Panda, A.K., Bisht, S.S., Mandal, S. and Kumar, N.S. 2016. Bacterial and archeal community composition in hot springs from Indo-Burma region, north-east India. ***Amb Express,*** *6*(1):111.

Rawat, N., and Joshi G.K. 2015. Prospecting for industrial enzymes in a hot spring metagenome. ***Journal of Pure and Applied Microbiology,*** *9*(2):1-5.

Riesenfeld, C.S., Schloss, P.D. and Handelsman, J. 2004. Metagenomics: genomic analysis of microbial communities. ***Annual Review of Genetics,*** *38*:525-552.

Rondon, M.R., August, P.R., Bettermann, A.D., Brady, S.F., Grossman, T.H., Liles, M.R., Loiacono, K.A., Lynch, B.A., MacNeil, I.A., Minor, C. and Tiong, C.L. 2000. Cloning the soil metagenome: a strategy for accessing the genetic and functional diversity of uncultured microorganisms. ***Applied and environmental microbiology,*** *66*(6):2541-2547.

Sahoo, R.K., Kumar, M., Sukla, L.B. and Subudhi, E. 2017. Bioprospecting hot spring metagenome: lipase for the production of biodiesel. ***Environmental Science and Pollution Research,*** *24*(4):3802-3809.

Sahoo, R.K., Subudhi, E. and Kumar, M. 2015. Investigation of bacterial diversity of hot springs of Odisha, India. ***Genomics data,*** *6*:188-190.

Saxena, R., Dhakan, D.B., Mittal, P., Waiker, P., Chowdhury, A., Ghatak, A. and Sharma, V.K. 2017. Metagenomic analysis of hot springs in Central India reveals hydrocarbon degrading thermophiles and pathways essential for survival in extreme environments. ***Frontiers in microbiology,*** *7*:2123.

Schmeisser, C., Krohn-Molt, I. and Streit, W.R. 2017. Metagenome Analyses of Multispecies Microbial Biofilms: First Steps Toward Understanding Diverse Microbial Systems on Surfaces. ***Functional Metagenomics: Tools and Applications***: 201-215.

Sharma, A., Jani, K., Shouche, Y.S. and Pandey, A. 2015. Microbial diversity of the Soldhar hot spring, India, assessed by analyzing 16S rRNA and protein-coding genes. ***Annals of microbiology,*** *65*(3):1323-1332.

Sharma, A., Paul, D., Dhotre, D., Jani, K., Pandey, A. and Shouche, Y.S. 2017. Deep sequencing analysis of bacterial community structure of Soldhar hot spring, India. ***Microbiology,*** *86*(1):136-142.

Singh, A., Subudhi, E., Sahoo, R.K. and Gaur, M. 2016. Investigation of the microbial community in the Odisha hot spring cluster based on the cultivation independent approach. ***Genomics Data,*** *7*:222.

Singh, R., Chopra, C., Gupta, V.K., Akhlaq, B., Verma, V. and Rasool, S. 2015. Purification and characterization of chpro1, a thermotolerant, alkali-stable and oxidation-resisting protease of Chumathang hotspring. ***Science Bulletin,*** *60*(14):1252-1260.

Singh, R., Dhawan, S., Singh, K. and Kaur, J. 2012. Cloning, expression and characterization of a metagenome derived thermoactive/thermostable pectinase. ***Molecular Biology Reports,*** *39*(8):8353-8361.

Staley, J.T. and Konopka, A. 1985. Measurement of in situ activities of nonphotosynthetic microorganisms in aquatic and terrestrial habitats. ***Annual Reviews in Microbiology,*** *39*(1):321-346.

Tanti, K.D. and Saha, S.K., 1993. Hydrobiological profiles along a thermal gradient of the hot springs of Rajgir(Bihar), India. ***Journal of Freshwater Biology,*** *5*(2):107-117.