

Review Article

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## Overview of Hot Water Springs and its Application

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### ABSTRACT

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This review starts with a basic introduction of geothermal springs definitions, classifications and its therapeutic values (Erfurt-Copper and Copper *et al.*, 2009; Eyton's Earth *et al.*, 2010; Erfurt *et al.*, 2011). Also include Global scenario of hot springs location of India as well as worldwide and its significances. Furthermore, it focuses on microorganisms' growth insight extreme environment and they are capable to produce various potential industrial compounds as such enzymes, antibiotics, hormones etc. and its having attraction in present scenario (Sing *et al.*, 2006; Nayak *et al.*, 2013). This chapter describes combination methods like basic conventional methods, advance molecular based techniques and also next generation sequencing. Lastly, this chapter discusses thermophilic microbial diversity of hot water springs in Gujarat, India and also other countries and its role as a various application in different area.

### Introduction

#### Hot Springs

A hot spring is a spring of geothermally heated ground water, rising up through the vents in the Earth's Crust. Geothermal springs are a natural source of underground heated water under pressure near the vicinity of active volcanic environments (Simoneit *et al.*, 2000; Allaby and Allaby 2003).

The hot spring waters contain huge amount of minerals (Fe, Mn etc.) and also have traces of gases (CO<sub>2</sub>, H<sub>2</sub>, H<sub>2</sub>S, CO) (Olive, 1990; Cown, 1992; Lengeler *et al.*,

1999; Resysenbach and Candy, 2001). The formation of hot water springs is shown in Figure 1.

Groundwater percolates through porous rock into fractures deep underground, where heat from a nearby magma chamber superheats the pressurized water to a temperature above the boiling point of water at surface pressure. In hot springs the rising superheated water is cooled below the boiling point by groundwater before reaching the surface. In geysers the superheated water collects in underground pockets. When the supply of steam and hot water is exhausted, the spouting stops and the cycles begin again. [Encyclopaedia Britannica Online.

<http://global.britannica.com/EBchecked/media/91382/Cross-section-of-a-geyser-and-hot-spring-Groundwater-percolates>. (Calderon, 2014)].

Geothermal springs have been defined and classified in various categories as shown in Table 1.

Traditionally, according to Indian mythology and Hindu beliefs hot springs have religious significance. Different types of minerals found in hot springs have different types of health benefits as shown in Table 2. Hot springs containing rich source of sulphur improve skin infections, skin inflammations and also respiratory problems (Eyton's Earth, 2010; Erfurt, 2011). According to the World Health Organization (WHO), thermal spring waters are believed to positively impact different kinds of disease and conditions of fatigue such as joint, kidney, and respiratory problems, however, such health alleviations need to be confirmed by further scientific studies. Some other common benefits attributed to soaking in hot springs include strengthening the immune system, improving skin conditions, enhancing blood circulation, promoting detoxification, and relaxing mind and body. In pursuit of these health benefits, all types of people visit hot springs to not only deal with health problems, but also as a preventative measure in maintaining good health and vitality (Erfurt, 2011).

Hot springs are located in many countries in the world, as indicated in the map (Figure: 2). Each hot spring has its own biological and geophysical characteristics, thus endowing them with fascinating potential applications for research, from the point of view of biotechnological and ecological subject areas (Brock, 1997; Tirawongsaraj *et al.*, 2008; Lau *et al.*, 2009; Klatt *et al.*, 2011; Briggs *et al.*, 2014; Prieto-Barajas *et al.*, 2017). Microbial communities can be found from the hot water spring based on temperature of the channel of the hot springs (Lengeler *et al.*, 1999).

There are many hot water springs located in India as well as in other countries. Several significance studies have been reported on hot water springs all over the world. They have been described in Table: 3, 4 and 5.

### **Thermal environment and thermophilic bacteria**

Earlier discovery made by Thomas D Brock in 1965 isolated *Thermus aquaticus* from hot water spring which are capable of producing thermostable Taq DNA polymerase (Brock and Freeze, 1969). The extremophiles

discovered during the earlier studies aroused an interest and also gave a challenging platform for the researchers. The extremophiles mainly include: halophiles, thermophiles, barophiles, psycrophiles and acidophiles. Microorganisms growing under the extreme conditions, produce valuable industrial compounds like enzymes, antibiotics, hormones etc. which have gained attention in the present scenario (Herbert and Sharp, 1992; Austin, 1998; Sing, 2006; Nayak, 2013). The largest popular thermal biotopes are hydrothermal systems that include volcanic and geothermal i.e. volcanic area which gives sulphurous gases and steam, the natural geothermal springs and the submarine hydrothermal vents. The hot solfataric fields are made up of two layers, upper layer and the lower layer. Upper layer is having vital amount of oxygen; it imitates an ochre colour due to the presence of the ferric ions; whereas the layer below displays a blackish-blue colour because of ferrous ions. Similarly, the submarine thermal systems consist of the hot springs, hot vents and sediments with very high temperatures (Stetter, 1998). The submarine hydrothermal vent system has high NaCl concentration and pH range between 5 to 8, exhibiting an acidic to alkaline pH (Horikoshi, 1998; Kikani, 2013).

Thermophiles are a type of extremophile which love extreme environments. Thermophilic bacteria survive at a higher temperature range and these microbes mostly have habitats in hot spring's environment. Most of the studies have been reported on thermophiles, found from diverse environmental habitat sources such as hot water springs and geothermal location (Panda *et al.*, 2013). Sub-category of thermophiles is divided based on their thermotolerance temperature: Facultative thermophiles can grow at 37° C and 50° C - 65° C, Obligate thermophiles have 65° C - 70° C temperature showing maximum growth and they don't grow below 50° C, extreme thermophiles grow at 40° C - 70° C with an optimal growth at about 65 °C temperature and Hyperthermophiles can grow over 90° C with an optimal temperature ranging between 80° C -115° C (Nayak, 2013). Thermophilic microorganisms can not only survive at a higher temperature but their growth rate and metabolic activities are also responsive at higher temperature (Tsuei – Yun Fang *et al.*, 2005).

Thermophiles microorganisms are prokaryotes (include aechea and bacteria) and eukaryotes, their protein, nucleic acid, cytoplasmic membranes allow them to grow in temperatures beyond 45° C. At a lower temperature thermophile's cellular components do not perform their

activity properly. On the other side, hyperthermophiles require over 80° C temperature for their optimal growth (Bauman, 2004). Thermophiles can survive in diverse habitats of high temperature, pH, salinity and extreme pressure (barophiles) (Kato, 2004). They were found to survive at 72° C (Kim *et al.*, 2001; Devi, 2015).

Thermophilic bacterial diversity provides their potential applications, which can be utilized for various purposes like, basic research knowledge and various biotechnological applications. Also study of thermophilic bacteria led to discovery of novel species (Huber *et al.*, 1991; Kozina *et al.*, 2010; Zhang *et al.*, 2010; Yohandini *et al.*, 2015). Due to their capability of the thermostability at a higher temperature thermophilic bacteria and their products have several potential applications in industries and also in biotechnological research processes, e.g. some enzymes (cellulases, amylases, proteases, pectinases, xylanases, lipases, esterases, catalases, gelatinases, keratinases, ureases, peroxidases, phytases, glucosidase and alcohol dehydrogenases etc.), proteins and bioactive compounds (Rekadwad, 2015; Rekadwad and Pathak, 2016).

### **Culture-dependent and Culture-independent diversity**

Bacterial classification within family, genus and species level depend on a broad range of phenotypic characterization (colony morphologies, Gram staining, endospore staining etc.). It gives details of the morphology of bacteria, conditions of the culture, appearance of the colony and their biochemical characterization (nutrient requirements - sugars, enzymatic activities, and /or metabolic activities) (Holt *et al.*, 1994).

Every new microbe requires biochemical analysis for its identification and metabolic activity. In India most of the laboratories depend on conventional methods for identification to find out the diversity of bacteria. During the 20<sup>th</sup> and 21<sup>st</sup> centuries, techniques for bacterial identification have undergone rapid growth. At the beginning, mostly conventional or phenotypic techniques were used (Petti *et al.*, 2005; Bailon-Salas *et al.*, 2017). Conventional method is used for bacterial classification and characterization based on their morphological, physiological and biochemical activities (Scow *et al.*, 2001). Based on the conventional methods, bacteria can be grouped into a number of natural assemblages depending on their characterization like shape, spore

forming / non-spore forming, aerobic / anaerobic, Gram-positive / Gram-negative (Sigeo, 2005). Classical methods like morphological and biochemical are used for identification and characterization of bacteria. It also includes isolation, laboratory culture and taxonomic characterization of individual (separate) bacterial species in environmental samples (Krishna, 2013).

Many microbial diversity studies have been done through culture-dependent and culture-independent approaches but researchers believe that traditional method approach, have many limitations in discovering a broad microbial diversity in the natural habitats. However, bacterial pure cultures are needed for their identification and characterization in understanding their physiology and at their genetical level (Ward *et al.*, 1998; Yohandini *et al.*, 2015).

In conventional methods, bacterial characterization is not steady, as it may be changed during stress conditions or evolution (Ocham *et al.*, 2005; Krishna, 2013). According to Verma *et al.*, in 2007, conventional techniques give information of only shape, size, colour, motility, staining properties, host-range, pathogenicity and assimilation of carbon sources and also these techniques do not provide a complete taxonomy of the microbes (Verma *et al.*, 2007). On the other hand, information on a successful concept of a microbial lineage (origin) is necessary to follow the complete approach. In the last few decades' appreciable development in the study of microbial taxonomy has taken place such as chemotaxonomy (Minnikin *et al.*, 1975), numerical taxonomy and DNA-DNA hybridization (Johnson, 1991), DNA amplification and sequencing (Konstantinidis and Tiedje, 2007) and whole genome sequencing (Janssen *et al.*, 2003; Das *et al.*, 2014). Thus, the use of a polyphasic approach, including a combined conventional and molecular based techniques is required to get a better knowledge of the bacterial diversity (Das *et al.*, 2014). Rastogi and Sani in 2011, mentioned that culture dependent traditional method can be allotted to only specific genera. Also, classical techniques take too much time for processing as well as depend upon the environmental conditions (Rastogi and Sani, 2011). Comparatively, the molecular based techniques are very fast, accurate with minor chances of error. The development of these advanced techniques has changed drastically, the definition of microorganisms as in many cases, sequence diversity within a species reveals multiple sequence clusters that are ecologically distinct (Cohan, 2002; Das *et al.*, 2014). 16S rRNA gene

techniques give information of phylogenetic information to identify culturable isolates at a genus level.

On the earth most of the microbial species are unculturable, means they are not capable to grow under the laboratory conditions (Lewis *et al.*, 2010). There is major limitation of utilization approach of culturable media. Based on the microscopic observation, in environment more than 99% microorganisms are not cultivable in the laboratory condition (Hugenholtz, 2002). It was mentioned by Rondon in 1999 that only 1% of the obtained cells can be studied in the microbial cultures as the probability of unculturable cells to be grown in culture under various conditions is limited. Many researchers goal has been focused on different advanced molecular techniques for the better understanding of the unculturable microorganisms (Rondon *et al.*, 1999; Bailon-Salas *et al.*, 2017). The 16S rRNA gene sequencing molecular-based techniques have been very fruitful for the phylogenetic relationship between various types of microorganism from different ecosystems (Cai *et al.*, 2003; Clarridge, 2004; Mignard *et al.*, 2006; Nayak, 2013). Advance molecular-based techniques and bioinformatics implementations have provided significantly unexplored microbial diversity. However, conventional and molecular-based methods have their own values and limitations. So, the concept of the combination of traditional and advance molecular techniques is the best way to find out the whole microbial diversity from the nature (Kikani, 2013).

## **Metagenomics Study**

Earlier reports on Yellowstone National Park hot spring were characterized by 5S rRNA sequencing from the mixed microbial flora and they found archaeobacterial and eubacterial species related to *Thermus species* population (Stahl *et al.*, 1985).

“Metagenomics” term was first time used by Jo Handelsman in 1998. The metagenomics study involves collection of genetic material directly from the environmental samples (Handelsman *et al.*, 1998). Metagenomic approach helps to find out culturable and unculturable microbes from the mixed microbial communities of environmental samples. Metagenomic study has given great knowledge and better understanding about evolution, lifestyle and diversity of the microorganisms from the hidden world of the microbes (Qin *et al.*, 2010; Hess *et al.*, 2011; Pignatelli and Moya, 2011; Mishra and Khanolkar, 2017).

Metagenomic study provides opportunity for yet to be cultivated 99% microbial diversity and their genetic material but still researcher’s final goal remains on microbial cultivation. The information given by metagenomics is used to flourish techniques of cultivation to isolate novel microorganisms and their products (Rondon *et al.*, 2000; Jiang *et al.*, 2011; Cai *et al.*, 2013; Gong *et al.*, 2013; Tan *et al.*, 2014; Grewal, 2014). The metagenomics process approach from the environmental samples has been shown in Figure 3.

Most of the microbial diversity are non-cultivable and it is very difficult to maintain bacterial pure culture in the laboratory condition, may be nutritional requirement and physical conditions like temperature, pH is responsible. During the process of culture-dependent method, sometimes it benefits to find microbial species, while on the other hand some microbes may be eliminated. Therefore, metagenomics (next generation sequencing) has in a way bypassed the bias of cultivation of the microbial strains by the enrichment techniques (Kikani, 2013). In Tanjung Sakti hot spring (South Sumatera), Indonesia, organisms have been cultivated and thermophilic bacteria have been identified through 16S rRNA sequencing and four distinct taxonomic groups: *Anoxybacillus*, *Geobacillus*, *Brevibacillus*, and *Bacillus* were found (Yohandini *et al.*, 2015).

Culture-independent metagenomics studies were done in Ganeshpuri (Nimbavali) hot spring, Mumbai region, Maharashtra, India. Proteobacteria, Firmicutes and Bacterioidetes were observed at a phylum level (Mishra and Khanolkar, 2017).

In the Tulsi Shyam hot spring, Gir Forest, Gujarat, India, thermophilic bacteria were isolated, identified, characterized and diversified using conventional method and Amplified Ribosomal DNA Restriction Analysis (ARDRA) approach. Based on conventional method, Gram-positive, rod shaped and catalase positive bacteria were obtained. Also molecular identification represented 3 Genera: *Anoxybacillus*, *Geobacillus/Aeribacillus* and *Bacillus* (Kikani *et al.*, 2015). Kikani in 2013 reported, culture-dependent seasonal diversity in Tulsi Shyam hot springs of Gujarat. A total of 64 bacterial strains were reported from water and soil samples. All the bacterial isolates exhibited Gram-positive features and were aerobic in nature. Catalase production was indicated positive in all of the isolates, whereas some of the isolates observed were oxidase positive. On the other hand, *Bacillus* and *Geobacillus* have been reported from

ARDRA profiles (Kikani, 2013). In Atri, Tarabalo and Taptapani hot springs of Odisha, Gram positive and rod shaped bacteria were observed with the help of traditional method and based on the molecular identification *Anoxybacillus*, *Geobacillus* and *Bacillus* species were observed (Nayak, 2013).

*Actinomycetes/thermoactinomycetes* were isolated and identified through biochemical tests and molecular method from Tulsi Shyam, Lasundra and TuvaTimba hot springs of Gujarat. All the isolates observed were Gram-positive and had large variation in Sugar profile (Shukla, 2015). Culture-independent study was done in Tulsi Shyam hot springs. In this metagenomic study (bacterial tag-encoded FLX amplicon pyrosequencing) most of the phyla indicated were Firmicutes, Proteobacteria and unclassified bacteria. Highest reported families were *Peptostreptococcaceae*, *Clostridiaceae* and *Enterobacteriaceae*. *Clostridium bif fermentans*, *Clostridium lituseburense* and uncultured ubiquitous bacterial species were obtained (Ghelani *et al.*, 2015). Aerobic thermophilic *Bacillus* genus was isolated using culturable method and also antimicrobial study has been done from Ma'en hot springs in Jordan (Fandi *et al.*, 2014). Sahay *et al.*, in 2017 reported 14 different types of genera *Anoxybacillus*, *Bacillus*, *Brevibacillus*, *Brevundimonas*, *Burkholderia*, *Geobacillus*, *Paenibacillus*, *Planococcus*, *Pseudomonas*, *Rhodanobacter*, *Thermoactinomyces*, *Thermobacillus*, *Thermonema* and *Thiobacillus*, which were found using 16S rRNA sequencing molecular-based method from Manikaran and Yumthang hot springs of Indian Himalayas (Sahay *et al.*, 2017). *Thermodesulfobacterium* (Thermophilic sulphate reducing prokaryote) were identified with both manual biochemical method and 16S rRNA sequencing molecular-based method from Vajreshwari and Ganeshpuri hot springs, Thane, Maharashtra (Patil *et al.*, 2014a).

Identification of thermophilic bacterial study was carried out based on cultural method from Ulu Legong hot spring in Kedah, Malaysia (Akmar *et al.*, 2011). Khalil *et al.*, in 2018 reported *Brevibacillus brostelensis* thermophilic strain which grew between 45° C and 70 ° C temperature by extended genomic analysis as well as biochemical based method (phenotypic characterization) from "Al-Ain Alhara" hot spring, located in the southeast of Gazan city in Saudi Arabia (Khalil *et al.*, 2018). *Tepidimonas taiwanensis* novel bacterial strain was isolated on the basis of phylogenetic analysis of 16S rDNA sequences, DNA-DNA similarity data, whole cell

protein analysis, fatty acid compositions as well as physiological and biochemical characterization from Sih-Chong-Si hot spring located in Pingtung county in southern Taiwan (Chen *et al.*, 2006). In Unkeshwar hot spring, Maharashtra, India, a total of 17 species were isolated based on morphological, biochemical and molecular analysis. These isolated strains belonged to Firmicute, Proteobacteria and Actinobacteria (Rekadwad and Pathak, 2016). Prieto-barajas *et al.*, in 2017 explored the culturable seasonal microbial diversity and physicochemical study on Tina and Bonita hot springs (Araro, Mexico). Total seventy-nine phylotypes were found using 16S rRNA sequences and they belong to Firmicutes, Proteobacteria and Actinobacteria phyla (Prieto-Barajas *et al.*, 2017).

In Manikaran hot spring, the bacterial diversity was investigated using culture-dependent method and it is predicted that it may be a maximum 0.1% of the total bacterial community (Ranjard *et al.*, 2000). In another study, foremost *Brevibacillus thermoruber*, *Paenibacillus sp.*, *Bacillus licheniformis*, *Bacillus pumilus*, *Bacillus subtilis*, *Pseudomonas psychrophila*, *Exiguobacterium acetylicum*, *Rhodococcus baikunurensis*, *Pseudomonas fluorescens*, *Bacillus megaterium* and *Staphylococcus succinus* thermophilic and thermotolerant bacteria were isolated and identified from Manikaram hot spring (Verma *et al.*, 2014; Kumar *et al.*, 2014a; Devi, 2015).

Podar and Resysenbach in 2006, discovered Taq polymerase (thermotolerant DNA polymerase) from the *Thermus aquaticus*, thermophilic bacteria in Yellowstone hot springs in the United State of America that is patented and thus Polymerase Chain Reaction gained its application throughout the world. These lead researchers to study on biodiversity of hot springs (Podar and Reysenbach, 2006).

Several studies on bacterial biodiversity have been conducted using culture-dependent as well as culture-independent techniques from Indonesian hot springs and Indonesian acidic hot spring, Kawah Hujan B, at Kamojang geothermal field, West Java hot spring (Baker *et al.*, 2001; Aminin *et al.*, 2008; Adiwati *et al.*, 2009).

From their findings most of the clones are associated to Crenarcheota and Proteobacteria from culture-independent study. On the other hand, those findings which were from culture-dependent analysis observed the influence of Firmicutes and gamma Proteobacteria Phylum.

**Table.1** Geothermal spring’s definition and general classification

<b>Classification Category</b>	<b>Definition</b>
<b>Geothermal Spring</b>	It includes hot springs and extreme hot springs, naturally heated while passing through underground voids and pore spaces.
<b>Natural Hot Spring</b>	It is a generic term for geothermal springs generally identified as, at least at body temperature and pleasant bathing temperature, mainly used for medicinal purposes and bathing.
<b>Thermal Spring</b>	It includes warm and hot springs generally above 25° C temperature, used for medicinal purposes and bathing.
<b>Mineral Spring</b>	It can be a cold, warm, hot, extremely hot or artificially heated spring, used for medicinal purposes and bathing.
<b>Thermo Mineral Spring</b>	It is a geothermal mineral spring water and artesian water used for medicinal purposes and bathing.
<b>Artesian Spring</b>	It is naturally discharging from subsurface with warm to hot water at 25° C to 100° C, used for medicinal purposes.
<b>Saline Spring</b>	It varies in temperatures, has a very high amount of mineral salt content, possible sea water used for medicinal purposes and bathing.
<b>Geyser</b>	It is an extremely hot spring, water reaches boiling point and above, a visual tourist attraction in geo tourism.
<b>Submarine Hot Spring</b>	Submarine vents known as black smokers emitting extreme hot water enriched with mineral and metallic trace elements.

Source: (Erfurt-Cooper and Cooper, 2009; Erfurt, 2011).

**Table.2** The therapeutic values of the mineral content of hot springs

<b>Minerals in hot springs</b>	<b>Their therapeutic values</b>
<b>Arsenic</b>	Limited amounts can assist the body with plasma and tissue growth. Foot bathing in mineral water with high content of arsenic, address fungal conditions of the feet.
<b>Bicarbonate</b>	Bicarbonate in hot springs can treat hypertension and mild atherosclerosis.
<b>Boron</b>	Boron is beneficial in building muscle mass, increasing brain activity and bones strengthening.
<b>Chloride</b>	It is beneficial for arthritis, rheumatic conditions, central nervous system conditions, posttraumatic and postoperative disorders, as well as orthopaedic and gynaecological disease.
<b>Magnesium</b>	Magnesium can convert blood sugar to energy and it also promotes healthy skin.
<b>Potassium</b>	It serves in the normalization of heart rhythms, assists in reducing high blood pressure, it also helps to eliminate body toxins and promotes healthy skin.
<b>Sodium</b>	Sodium and natural salts help in arthritic symptoms and it can stimulate the body’s lymphatic system when bathed in hot springs.
<b>Sulphur and Sulphates</b>	Sulphur is a rich in Hot Springs. It treats skin infections, respiratory problem and skin inflammations.

Source: (Eyton's Earth, 2010; Erfurt, 2011).

**Table.3** List of Indian hot springs

Hot springs	States
TuvaTimba, Tulshishyam, Unai, Lasundara, Dholera	Gujarat
Vajreshwari, Ganeshpuri, Akloli, Aravali	Maharashtra
Tatapani, Dhuni Pani, Chavalpani, Anthoni, Babeha	Madhya Pradesh
Ushnagudam, Rudrakund	Andhra Pradesh
Mannargudi	Tamil Nadu
Bendrutheertha, Bandaru, Irde	Karnataka
Tattapani, Tatwani, Manikaran, Khirganga, Vashisth, Jeori, Kasol, Kalath	Himachal Pradesh
Suryakund, Gaurikund, Taptkund, Rishikund	Uttarakhand
Yumthang, Borang, Phurchachu (Reshi), Ralang, Yumey Samdong, Taram – chu	Sikkim
Rameshkund, Lakshmikund, Tatal Pani, Janamkund, Shringshrishi, Rajgir, Sitakund, Bhaduri Bhur	Bihar
Atri, Taptapani, Tarabalo, Deulajhari	Orissa
Tantloi, Tantni, Bakreshwar of Birbhum, Kendughata, Bholeghata	West Bengal
Hot spring of Dirang area	Arunachal Pradesh
Tatta, Ram Kund, Brahma Kund, Jaron	Jharkhand
Chumathang, Panamik	Ladakh, Jammu and Kashmir
Jakrem	Meghalaya

**Table.4** Location of hot springs in India and their significance

No.	Hot springs	Location	Significance
1	TuvaTimba	Gujarat	Isolated biosurfactant producing bacteria ( <b>Joshi et al., 2013</b> ). Thermophilic thermotolerant actinomycetes isolated for diversity phylogeny and biocatalytical potential ( <b>Shukla, 2015</b> ). Reported metagenomics study using shotgun sequencing approach ( <b>Mangrola et al., 2015a</b> ). Seasonal thermophilic microbial diversity has been studied ( <b>Patel et al., 2019a</b> ).
2	Dholera	Gujarat	Isolation, screening and characterization of alkaline protease producing microorganisms ( <b>Chudasama, 2012</b> ). Geothermal exploration case study was carried out ( <b>Sircar et al., 2015</b> ). Culturable thermophilic microbial diversity found out ( <b>Patel et al., 2019b</b> ).
3	Tulsi Shyam	Gujarat	<i>Bacillus amyloliquifaciens</i> strain was isolated and characterized and purified of a thermostable and calcium independent $\alpha$ amylase from this strain ( <b>Kikani and Singh, 2011</b> ). Studies have been focused on molecular based diversity and biocatalytic perspectives of thermophilic and thermotolerant bacteria ( <b>Kikani, 2013</b> ). Thermophilic thermotolerant actinomycetes isolated for diversity phylogeny and biocatalytical potential ( <b>Shukla, 2015</b> ). Culture dependent diversity has been studied ( <b>Kikani et al., 2015</b> ). Amplicon pyrosequencing based metagenomics study was done ( <b>Ghelani et al., 2015</b> ). Studied alkaline protease producing thermotolerant alkaliphilic isolates ( <b>Patel et al., 2006</b> ).
4	Lasundra	Gujarat	Isolated biosurfactant producing bacteria ( <b>Joshi et al., 2013</b> ).

			Produced , purified and immobilized extracellular lipases Xrf11 strain and <i>Bacillus licheniformis</i> Xrf12 strain used for production of alkyl esters (Gohel <i>et al.</i> , 2013). First study reported on metagenomics using Ion Torrent PGM platform (Mangrola <i>et al.</i> , 2015b). Thermophilic thermotolerant actinomycetes isolated for diversity phylogeny and biocatalytical potential (Shukla, 2015).
5	Unai	Gujarat	<i>Bacillus licheniformis</i> , thermophilic bacteria was isolated and characterized and its applications on thermostable and solvent-tolerant serine protease (Dudhagara <i>et al.</i> , 2014). <i>Bacillus cohini</i> , thermophilic alkaline protease producing strain was isolated (Dudhagara <i>et al.</i> , 2015). Ion Torrent based metagenomics approach was used to find out microbial diversity (Mangrola <i>et al.</i> , 2018).
6	Ganeshpuri	Maharastra	Isolated and characterized <i>Thermodesulfobacterium</i> and its application in bioremediation process (Sulphate reduction) (Patil <i>et al.</i> , 2014a). Isolated thermophilic bacillus species with extracellular enzymatic activities and that can be exploited for biotechnological purpose (Lele and Deshmukh, 2016). Microbial diversity identified through metagenomics (Mishra and Khanolkar, 2017). Isolated and purified bacteria from hot spring's soil samples (Pednekar <i>et al.</i> , 2011).
7	Unkeshwar	Maharastra	Physicochemical and microbial studies have been done (Bhusare and Wakte, 2011). Studied water quality using Physical, chemical, ionic, biological parameter (Yannawar <i>et al.</i> , 2013). Investigated microbial diversity based on metagenome sequencing (Mehetre <i>et al.</i> , 2016).
8	Akoli	Maharastra	Thermophilic <i>Actinomycetes</i> isolated that are capable of producing industrial enzymes (Chaudhary and Prabhu, 2016).
9	Vajreshwari	Maharastra	Isolated biosurfactant producing bacteria (Joshi <i>et al.</i> , 2013). Isolated and characterized <i>Thermodesulfobacterium</i> and its application in bioremediation process (Sulphate reduction) (Patil <i>et al.</i> , 2014a). Thermophilic <i>Actinomycetes</i> isolated that are capable of producing industrial enzymes (Chaudhary and Prabhu, 2016).
10	Tarabalo	Odisha	Isolated thermophilic bacteria and it was active for amylase enzyme (Khuntia <i>et al.</i> , 2010). Found true thermophiles and could be a source of thermostable protease enzymes, which can be used for pharmaceuticals and industrial applications (Panda <i>et al.</i> , 2013). Isolated thermophilic microorganisms with the help of conventional and molecular based method. Determined heavy metal removal and amylase activity (Nayak, 2013).
11	Taptapani	Odisha	Lipolytic thermophilic organisms were characterized (Panda, 2011). Isolated thermophilic microorganisms with the help of conventional and molecular based methods. Determined heavy metal removal and amylase activity (Nayak, 2013).
12	Atri	Odisha	Isolated thermophilic microorganisms with the help of conventional and molecular based methods. Determined heavy metal removal and amylase activity (Nayak, 2013). They have studied a natural ecosystem for global warming research (Mohanty, 2014).
13	Vashist	Himachal Pradesh	Culturable microbial diversity of thermophilic bacteria was found to explore their potential industrial applications (Sharma <i>et al.</i> , 2013).

			Evaluated thermotolerant bacteria using culturable method ( <b>Kumar et al., 2014b</b> ). Pigment producing bacteria were focused for their industrial applications ( <b>Meenakshi, 2015</b> ). Isolated and characterized thermophilic bacteria ( <b>Shirkot and Verma, 2015</b> ).
14	Tattapani	Himachal Pradesh	Xylanase producing thermophilic bacteria isolated and characterized ( <b>Chauhan, 2010</b> ). Culturable microbial diversity of thermophilic bacteria found to explore their potential industrial applications ( <b>Sharma et al., 2013</b> ). Pigment producing bacteria were focused for their industrial applications ( <b>Meenakshi, 2015</b> ). Microbial diversity using metagenomic approach ( <b>Mahajan et al., 2016</b> ).
15	Manikaram	Himachal Pradesh	Xylanase producing thermophilic bacteria isolated and characterized ( <b>Chauhan, 2010</b> ). Culturable microbial diversity of thermophilic bacteria found to explore their potential industrial applications ( <b>Sharma et al., 2013</b> ). Pigment producing bacteria were focused for their industrial applications ( <b>Meenakshi, 2015</b> ). Isolated and characterized thermophilic bacteria ( <b>Shirkot and Verma, 2015</b> ). Isolated, identified and characterized aerobic thermophiles to find out important thermostable enzymes from potential isolates ( <b>Devi, 2015</b> ).
16	Khiganga	Himachal Pradesh	Isolated and characterized thermophilic bacteria ( <b>Shirkot and Verma, 2015</b> ) .
17	Kasol		
18	Kalath		
19	Bakreshwar	West Bengal	Culture-independent approach has been used to investigate microbial diversity ( <b>Ghosh et al. 2003</b> ). Cellulase activity by isolated thermophilic bacteria ( <b>Acharya and Chaudhary, 2011</b> ). Isolated, characterized and molecular identified esterase producing thermophilic bacteria, has the ability of tributyrin degradation ( <b>Ghati et al., 2013</b> ). Evaluated thermotolerant bacteria using culturable method ( <b>Kumar et al., 2014a</b> ). Novel bacterium (BKH3) has been identified from hot spring water samples consortium ( <b>Chaudhuri et al., 2016</b> ).
20	Metaldanga	West Bengal	Novel bacterial strain was isolated and identified. This strain has application at industrial level and synthesis of nanoparticles ( <b>Alam et al., 2016</b> ).
21	Yumthang	Sikkim, Himalayas	Bacterial community found using Illumina platform (metagenomics) ( <b>Panda et al., 2016</b> ). Illumina sequencing platforms were used for bacterial metagenomics study ( <b>Panda et al., 2017</b> ). Diverse thermoactive enzymes were detected to be used for potential applications ( <b>Sahay et al., 2017</b> ).
22	Mani-karan	Himalyas	Diverse thermoactive enzymes were detected to be used for potential applications ( <b>Sahay et al., 2017</b> ).
23	Anhoni	Madhya Pradesh	Microbial community found through metagenomic analysis and the presence of chemoorganotrophic thermophilic community with the

			ability to utilize complex hydrocarbons as a source of energy ( <b>Saxena et al., 2017</b> ).
24	Surajkund	Jharkhand	Find out amylase, xylanase and cellulose producing bacteria through the culture-dependent and culture-independent techniques ( <b>Soy et al., 2023</b> ).
	Laxmankund		
	Brahmakund		
	Ramkund		
	Sitakund		
	Mixedkund		
24	Jakrem	Meghalaya	Bacterial community found using Illumina platform (metagenomics) ( <b>Panda et al., 2016</b> ).
25	Tattapani	Sarguja, Chhattisgarh	Microbial community found through metagenomic analysis and the presence of chemoorganotrophic thermophilic community with the ability to utilize complex hydrocarbons as a source of energy ( <b>Saxena et al., 2017</b> ).

**Table.5** Global hot water springs other than India and their significance.

No.	Hot springs	Location	Significance
1	Yellowstone	USA	Case study was done on chemical analysis of thermal water ( <b>Morey, 1973</b> ). Thermophilic bacteria were characterized ( <b>Marsh and Don, 1953</b> ). Advanced Spaceborne Thermal Emission and Reflection Radiometer (ASTER) and the Airborne Visible/IR Image Spectrometer (AVIRIS) data were used to characterize hot spring deposits in the Lower, Midway, and Upper Geyser Basins of Yellowstone National Park from the visible/near infrared (VNIR) to thermal infrared (TIR) wavelengths ( <b>Hellman and Michael, 2004</b> ). Presented bacterial diversity analysed by metagenomics from globally distributed terrestrial hot springs ( <b>Menzel et al., 2015</b> ).
2	Cahuelmo	North Patagonia, Chile (South America)	Microbial diversity, microheterogeneity and biogeography found out hot spring microbial mats ( <b>Calderon, 2014</b> ).
3	Procelana		
4	Procelana geyser		
5	Elegedi		
6	Akwar	East Africa	Investigated pathogenic bacteria with the help of high-throughput sequencing technologies ( <b>Ghilamicael et al., 2018</b> ).
7	Maiwooi		
8	Garbanabra		
9	Gelti		
10	Ain Allah		
12	Abaynou	Moroccan, North Africa	Thermophilic bacteria investigated and those strains are having important enzymes protease and amylase ( <b>Tarik et al., 2015</b> ).
13	Moulay Yaacoub		
14	Ain Jerri		
15	Siloam	Limpopo, South Africa	First metagenomic bacterial diversity was done using 454 pyrosequencing ( <b>Tekere et al., 2011</b> ).
			Study has been done in metagenomics analysis of bacterial diversity ( <b>Tekere et al., 2015</b> ).

16	Die Eiland	Limpopo, South Africa	Bacterial metagenomics study has been done ( <b>Tekere et al., 2015</b> ).
17	Souting		
18	Tshipise		
19	Mphephu		
20	Sagole		
21	Sungai Tutung	Kerinci, Jambi (Indonesia)	Different thermophilic strains were identified and all these strains produced potential alkali proteases enzyme ( <b>Arzita et al., 2017</b> ).
22	Tanjung Sakti	South Sumatera (Indonesia)	Isolated thermophiles and also indicated phylogenetic analysis and clustered within distance of taxonomic groups of microbes ( <b>Yohandini et al., 2015a</b> ).
23	Cimanggu	Indonesia	Described bacterial classification using molecular based method directly amplified from the hot springs and also consortia ( <b>Baker et al., 2001</b> )
24	Cibuni		
25	Domas		
26	Levunovo	Bulgaria, Europe	Microbial diversity investigated and 16S rRNA and GH-57 two genes explored ( <b>Stefanova et al., 2015</b> ) .
27	Vetren Dol		
28	Pozzuoli	Italy	Presented bacterial diversity analysed by metagenomics from globally distributed terrestrial hot springs ( <b>Menzel et al., 2015</b> ).
29	Pisciarelli		
30	Bagnaccio	Viterbo, Italy	Isolated and identified a moderately thermophilic anaerobic bacteria from mixed sediments/water samples and also got novel species <i>Caloramator viterbensis</i> ( <b>Seyfried et al. 2002</b> ).
31	Antun	Taiwan	A slightly thermophilic bacterial strain, <i>Tepidimonas fonticaldi</i> species isolated and characterized using a polyphasic taxonomic approach ( <b>Chen et al., 2013</b> ).
32	Rahsawarin	Thailand	Described bacterial count, physico-chemical analysis, metal analysis, ions and nutrients were measured ( <b>Sudjaroen et al., 2018</b> ).
33	Porn-Rang		
34	El Carrizal	Veracruz, Mexico	Isolated and identified thermophilic and alkalitolerant bacterial strains and the strains have capacity to produce hydrolytic enzymes under thermophilic conditions ( <b>Pinzón-Martínez et al., 2010</b> ).
35	Los Banos		
36	Lake Magadi	Kenya	Studied illumina sequencing and amplicon analysis of both total community rDNA and 16S rRNA, cDNA to determine the bacterial and community structure of bacteria and archaea ( <b>Kambura et al., 2016</b> ).
37	Little Magadi		
38	Grensdalur	Iceland	Presented bacterial diversity analysed by metagenomics from globally distributed terrestrial hot springs ( <b>Menzel et al., 2015</b> ).
39	Krisuvik		
40	Tibetan Plateau	Northwest China	Represented work on biodiversity of thermophilic bacteria ( <b>Wang et al., 2013</b> ).
41	Tengchong	Yunnan Province, China	Temporal variation was observed in microbial diversity ( <b>Huang, 2014</b> ).

42	Eryuan	China	Microbial diversity analysed based on metagenomics approach (Menzel <i>et al.</i> , 2015). Tangchi and Bantang hot spring water samples collected in Hefei, China. Illumina MiSeq system was utilized to sequence and analyze the bacterial 16S rRNA gene (Zhang <i>et al.</i> , 2023).
43	Benguet	Philippines	First study was carried out to characterize thermophilic bacteria based on culture-dependent method and molecular method (Socorro <i>et al.</i> , 2015).
44	Tatta Pani	Azad Kashmir, Pakistan	Facultative anaerobic bacteria were isolated and identified based on molecular method (Zahoor <i>et al.</i> , 2012). Isolated and identified unexplored thermophilic bacteria with the help of morphological, biochemical, physiological and molecular attributes (Ishaq <i>et al.</i> , 2023).
45	Uzon Caldera	Kamchatka peninsula, Russia	Isolated and characterized nitrite-oxidizing moderately thermophilic bacteria (Lebedeva <i>et al.</i> , 2011). Metagenomics studies have been done and also novel genes identified which are used for lipolytic and proteolytic enzyme activity (Wemheuer <i>et al.</i> , 2013). Bacterial diversity identified using metagenomic method (Menzel <i>et al.</i> , 2015). Investigated Aerobic, chemolithoautotrophic, hydrogen- and sulfur-oxidizing thermoacidophilic bacteria dominated the microbial population in the hydrothermal water and are principal producers of organic matter (Maltseva <i>et al.</i> , 2023).
46	Mutnovsky volcano	Kamchatka peninsula (Russia)	Novel gene identified based on metagenomics analysis and used for lipolytic and proteolytic enzyme activity (Wemheuer <i>et al.</i> , 2013).
47	Gorjachinsk	Buryat Republic, Russia	Isolated and characterized nitrite-oxidizing moderately thermophilic bacteria (Lebedeva <i>et al.</i> , 2011).
48	Neungam carbonate	Republic of Korea	In this study, Identification was done based on the full-length 16S rRNA gene sequence (Lee <i>et al.</i> , 2022).
49	Mungang sulfur		
50	Deokgu		
51	Baegam		
52	Dongnae		
53	Sungai Klah	Malaysia	Identified thermophilic diversity using shotgun metagenomics platform (Chan <i>et al.</i> , 2015).
54	Arzakan	Armenia, Eurasia	Microbial diversity studied using molecular and culture-based techniques (Panosyan and Nils-Kare, 2014).
55	St. Lucia	Sou-friere, Eastern Caribbean, West Indies	Founded geochemistry and microbial diversity correlations (Stout <i>et al.</i> , 2018).
56	Onikobeonsen-kyo	Miyagi, Japan	Isolated highly thermostable $\beta$ -xylosidases enzymes from a soil microbial community of hot spring using a metagenomic approach (Sato <i>et al.</i> , 2017).
57	Pamukcu	Balikesir, Turkey	Identified and characterized thermophilic bacteria with the help of phenotypic and genotypic methods including molecular based 16S rRNA sequencing, repPCR (Repetitive element sequence – based PCR) profile and fatty acid methyl ester (Adiguzel <i>et al.</i> , 2009).
58	Llica	Erzurum, Turkey	
59	Akdag	Erzurum, Turkey	
60	Sorgun	Yozgat, Turkey	

61	Pasinler	Erzurum, Turkey	Studied thermophilic bacterial identification and characterization ( <b>Adiguzel et al., 2011</b> ).
62	Karakoc	Izmir, Turkey	Novel moderately thermophilic, <i>Brevibacillus aydinogluensis</i> species was isolated ( <b>Inan et al., 2012</b> ).
63	Sındırgı	Balıkesir, Turkey	First reported study on microbial diversity using culture-dependent and culture-independent methods ( <b>Cankili, 2016</b> ).
64	Havran		
65	Güre	Eastern and Southeastern Anatolia Regions of Turkey	Isolated and identified bacteria with 16S rRNA sequencing ( <b>Ulucay et al., 2022</b> ).
66	Eynal		
67	Kopru (Agri)		
68	Hista (Siirt)		
69	Hasanabdal (Van)		
70	Davut (Agri)		
71	Guclukonak (Sirnak)		
72	Dargecit (Mardin)	Jordan (Middle East)	Culture-dependent and Culture-independent bacterial diversity studies have been done ( <b>Malkawi and Al-omari, 2010</b> ). Thermophiles study has been done ( <b>Fandi et al., 2014</b> ). Explored microbial diversity using metagenomic analysis ( <b>Hussein et al., 2017</b> ). Indicated new chemicals, microbiological data, antimicrobial activity and heavy-metals analysis ( <b>Shakhatreh et al., 2017</b> ). Studied antimicrobial activity of <i>Streptomyces</i> strains ( <b>Abussaud and Alanagreh, 2013</b> ). Isolated thermophilic bacteria and they showed ability to produce thermostable enzyme ( <b>Mohammad et al., 2017</b> ).
73	Pasinler (Erzurum)		
74	Afra		
75	Ma'in		
64	Ashounah		
65	Waggas		
66	Zara		
67	Alshounch		
68	Waggas		
69	Al-Mansheyah		
70	Deir Alla		
71	Hammamat		
72	Zara Dead Sea		
73	Hammamt Afra	Jazan (Saudi Arabia)	Studied thermophilic bacteria and highlighted their capability for the production of amylolytic and proteolytic enzymes ( <b>El-Gayar et al., 2017</b> ).
74	Al-Burbita		
75	Al-Hemma	Shri lanka	Illumina MiSeq sequencing of the V3–V4 region of the 16S rRNA gene. heat-stable protease and amylase producers, nitrogen-fixing organisms, and potential bioremediation bacterial species, were detected in all the wells. ( <b>Rupasinghe et al., 2022</b> ).
76	Bani Malik		
77	Rankihiriya		
78	Kanniya		
79	Nelum-wewa,		
80	Mahaoya		
81	Wahawa	Madunagala	
82	Madunagala		

**Figure.1** Formation of hot water springs [(Source: (Calderon, 2014)].

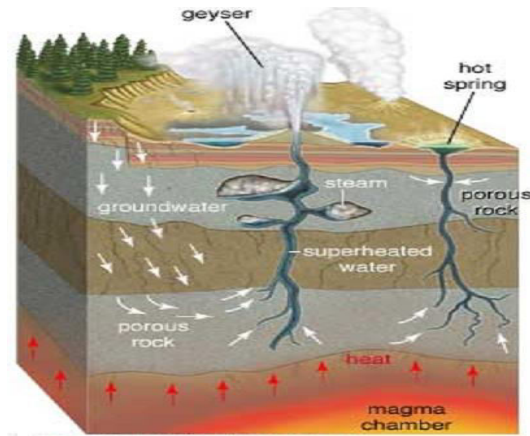
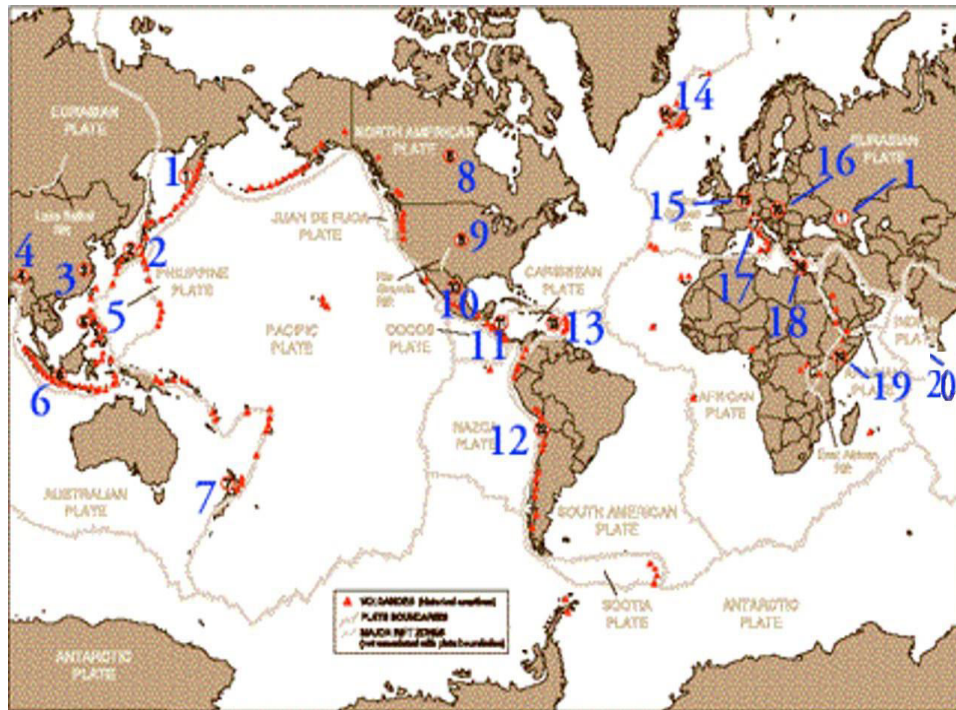
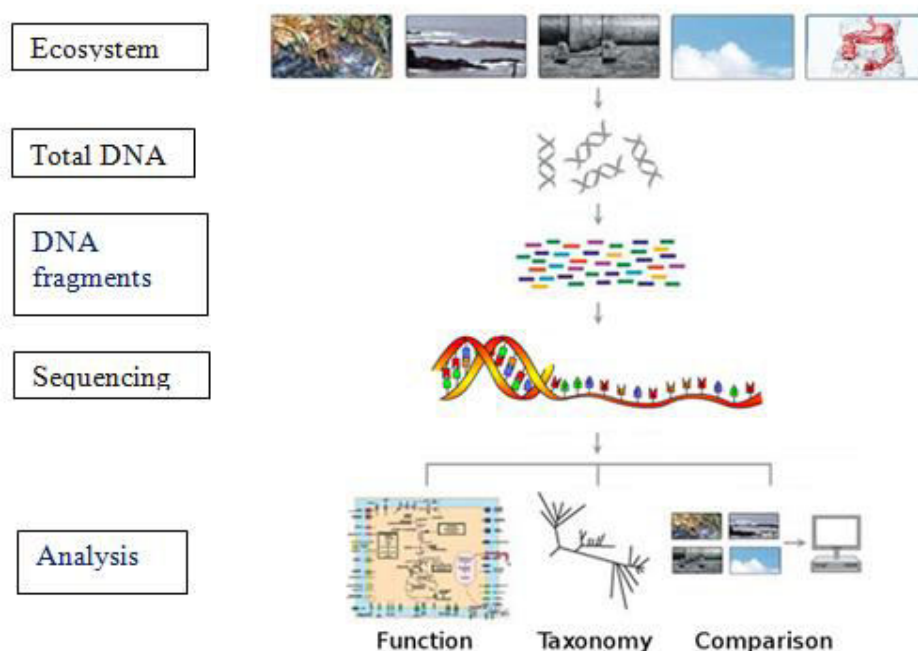


Figure.2 Worldwide location of hot springs ([http://geothermal.marine.org/geomap\\_1.html](http://geothermal.marine.org/geomap_1.html))



- |                              |  |
|------------------------------|--|
| 1. Russia                    | 11. Central American Volcanic Belt     |
| 2. Japan                     | 12. Andean Volcanic Belt               |
| 3. Eastern China             | 13. The Caribbean Islands              |
| 4. Himalayan Geothermal Belt | 14. Iceland and other Atlantic Islands |
| 5. The Philippines           | 15. Northern Europe                    |
| 6. Indonesia                 | 16. Eastern Europe                     |
| 7. New Zealand               | 17. Italy                              |
| 8. Canada                    | 18. Eastern and Southern Mediterranean |
| 9. United States             | 19. East Africa Rift System            |
| 10. Mexico                   | 20. India                              |

Figure.3 Pipeline of Metagenomics Study (Ghazanfar *et al.*, 2010).



The presence and abundance of numerous kinds of extremophiles inside severe habitats can be revealed through amplicon sequencing of extreme locations. 16S rDNA amplicons from soil and water samples from Tuva Timba and water samples from Dholera in Gujarat, India, were utilized for identifying Firmicutes, Proteobacteria, and Actinobacteria as significant taxa. There was less diversity in the season-wise data (Patel *et al.*, 2020(a)).

In Bakreshwar hot springs microbial diversity was estimated by a culture-independent method (Malhotra *et al.*, 2000; Ghosh *et al.*, 2003).  $\gamma$ -Proteobacteria, cyanobacteria and green non-sulphur and low-GC Gram-positive bacteria were observed from 16S rDNA clones from sediment samples.

*Geobacillus thermooleovorans* thermophilic strains were isolated and identified from hot water spring (Sharma, 2000) and also effluent samples from paper pulp industry (Kashefi and Lovely, 2003). In the present study, physicochemical and microbiological diversity assessments of seven Indian hot springs |Ganeshpuri, Bendru Theertha, Tuwa, Vajreshwari, Akaloli, Sativali and Bhadrachalam. were undertaken. Culture-dependent and culture-independent microbial diversity analyses (Narsing Rao *et al.*, 2021).

### **Applications of Thermophilic bacteria**

Thermophilic bacteria which mostly survive in high temperature are located in hot springs. Due to the thermophilic bacterial growth at high temperatures and their novel macromolecular properties, thermophilic bacteria have high metabolism, stable enzymes and higher yield of end products compared to the similar type of mesophilic species (Haki and Rakshit, 2003). Hot springs are a source of potential microorganisms that have the capacity to produce hydrogen (H<sub>2</sub>) and ethanol. It was presumed by researchers that thermophilic bacteria are strongly capable of hydrogen production and cellulose degradation rather than mesophilic bacteria. Especially the amount of cellulolysis is assumably faster at a higher temperature (Wiegel and Ljungdahl, 1986; Blumer-Schuetz *et al.*, 2008). It has been reported in 1998, in biological process, contamination risk factor by other organisms became less, where high temperature are necessary (Adams and Kelly, 1998).

Bioethanol product can also be obtained from thermophilic bacteria and it has numerous advantages like, distillation process is highly efficient at high temperature incubation period, do not require cooling process and some of the thermophiles have capability to direct fermentation of polysaccharides to ethanol. These type of properties of thermophilic bacteria indicate that they are better alternative source to yeast (Gough *et al.*, 1996).

Thermophiles also have potential and productive applications in various fields such as biomining, bioenergy, biosurfactant and thermozyne (Urbetia *et al.*, 2015). Many reported studies mentioned that anaerobic thermophilic bacteria have ability to produce biofuels (Canganella and Wiegel, 2014). Use of thermophilic bacteria in Consolidated Bioprocessing (CBP) offers a successful substitute for production of biohydrogen (Parisutham *et al.*, 2014). Reported studies have observed that moderate thermophiles microorganisms have best application for biomining and commercially applications of biomining process run below temperature at 40-50° C (Donati and Sand, 2007). Thermophilic bacteria can grow at high temperature and at high temperature rate of bioleaching process has been increased (Urbetia *et al.*, 2015). Thermophilic *Aeribacillus* and *Geobacillus* bacteria have been reported for bioremediation capability for many hydrocarbons and aromatic compounds (Mnif *et al.*, 2014). Reported studies have shown that *Anoxybacillus*, thermophilic strains have ability to degrade dye (Deive *et al.*, 2010). Thermophilic bacterial strains such as *Aneurinibacillus*, *Geobacillus*, *Alcaligenes*, *Bacillus* and *Brevibacillus* have been reported for higher production of biosurfactants (Bharali *et al.*, 2011; Joshi *et al.*, 2008; Mnif *et al.*, 2011; Sharafi *et al.*, 2014; Urbetia *et al.*, 2015).

*Clostridium thermocellum*, *Clostridium stercorarium*, *Clostridium straminisolvens*, *Caldicellulosiruptor saccharolyticus*, and *Caldicellu-losiruptor obsidiansis* anaerobic thermophilic bacteria have capacity to utilize cellulose (Madden, 1983; Wiegel and Dykstra, 1984; Freier *et al.*, 1988; Rainey *et al.*, 1994; Kato *et al.*, 2004; Hamilton-Brehm *et al.*, 2010). Verma and Shirkot in 2014 purified laccase enzyme from *Geobacillus thermocatenulatus*, thermophilic species and it can be effectively used in removal of the industrial dyes which can cause environmental pollution (Verma and Shirkot, 2014). Thermophilic *Bacillus* species have a potential application in environment pollution degradation. Indigenous thermophilic hydrocarbon degraders are of special significance for the bioremediation of oil-polluted desert soil (Margesin and Schinner, 2001). Nowadays due to the industrial activity metal contamination problem is increasing day by day, and microorganisms when in contact with heavy metals can decrease metal solubility and mobility. The metal and sulphate-reducing bacteria have suitable physiology for metal precipitation and immobilization. The activities of these microbes provide metabolic products such as iron and hydrogen

sulphide, which lead to mineral formation. These minerals can react with heavy metals, resulting in precipitation and hence detoxification (Chalaal and Islam, 2001; Mehta *et al.*, 2016).

This study uses thermophilic bacteria obtained from Gujarat's Tuva Timba and Dholera hot springs to demonstrate the degradation of low density polyethylene (LDPE) (Patel *et al.*, 2020(b)).

Gram-positive strains of *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus sonorensis*, and Gram-negative strains of *Tepidimonas taiwanesis* were chosen for biofilm production in this investigation. By employing the 96-well microtiter plate technology, the quantification of biofilm development and enhanced capacity were examined. Additionally, examine the impact on biofilm formation by adding a carbon source, a nitrogen source, and a combination of inhibitors, such as sodium salicylate and thioglycolate (Patel *et al.*, 2021).

Bakreshwar hot spring in West Bengal, India could be a source of cellulase activities and bacteria are found as a novel source of endoglucanases and exoglucanase fuction at higher temperature (Acharya and Chaudhary, 2011).

Previous study from Jordan thermal springs, mentioned thermophilic bacteria have a great antimicrobial activity and also indicated that *Bacillus* strains may be a good producer of antibiotics and also have antimicrobial effects against Gram-positive bacteria at higher temperature (Fandi *et al.*, 2014).

The three highly populated nations of China, India, and Pakistan are surrounded by the Himalayan Geothermal Belt (HGB), which is home to a large number of hot springs. Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria are the most common bacterial phyla found in the hot springs found throughout these countries, according to studies on the subject. There is a noteworthy positive link between the diversity of bacteria and physicochemical parameters such as pH, temperature, Na<sup>+</sup>, HCO<sub>3</sub><sup>-</sup>, and so on. The water in these hot springs is primarily of the Na-Cl, Na-HCO<sub>3</sub>, SO<sub>4</sub>-Cl, and mixed types, according to physicochemical investigations. The temperature ranges between 100 and 250°C, as indicated by different geothermometers (Nabi Najjar *et al.*, 2022). The present study examined the variety of thermophilic microorganisms found in bacteria in thermal hot springs located in the Indian Himalayan Region (IHR). It is also noted that the development of

thermophilic bacteria isolated from IHR's thermal hot springs occurs at temperatures between 40 and 100 °C and pH values between 3.5-8, producing a variety of enzymes and metabolites that have been documented in literature through metagenomics research (Verma *et al.*, 2022).

### Author Contributions

Patel Disha: Investigation, formal analysis, writing—original draft. Dave Shailesh: Validation, methodology, writing—reviewing. Antony Suresh:—Formal analysis, writing—review and editing. Braganza Vincent: Investigation, writing—reviewing. Modi Hasmukh: Resources, investigation writing—reviewing.

### Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Declarations

**Ethical Approval** Not applicable.

**Consent to Participate** Not applicable.

**Consent to Publish** Not applicable.

**Conflict of Interest** The authors declare no competing interests.

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