Hot and Cold Bacteria of Sikkim: Biodiversity and Enzymology

13

Sayak Das, Mingma Thundu Sherpa, Ishfaq Nabi Najar, and Nagendra Thakur

Abstract

The obnubilated macrocosm of microbes are influential, herculean, and is an enigma to us “the humans”—supposedly the wisest species to be evolved ever. The insane ability of microorganisms to survive, adapt, and utilize or metabolize, on every possible nook on earth is like a whodunit and supreme mastery. Their hejira from humans and also the ability to “live-out and live within” us hoodwinking our defence system is shrouded and veiled. In, Sikkim, the north-eastern state of India, diversified micro-flora, and fauna thrive in almost the entire landscape. It is a privilege to have both the extreme conditions of life here at Sikkim—HOT and COLD! The glacier hosts psychrophiles and the hot spring incubates thermophiles. Thus, the exploration of extremozymes from both these special ecology is one of our prime research interests. In this chapter, we have discussed briefly about our research findings on bacterial diversity at hot springs and glaciers of Sikkim. Among hot springs, we have discussed our studies from Borong, Dzongu, Polok, Reshi, Yumthang, and Yume Samdung whereas among glaciers, we have focused on Changme Khang, Changme Khangpu, Chumbu, and Kanchengayao. Some potential bacteria as polyextremophiles have also been highlighted.

Keywords

Thermozyme · Cryozyme · Hot spring · Glacier · *Geobacillus* · *Pseudomonas* · Sikkim

---

S. Das · M. T. Sherpa · I. N. Najar · N. Thakur (✉)
Department of Microbiology, School of Life Sciences, Sikkim University, Gangtok, Sikkim, India
e-mail: nthakur@cus.ac.in

H. Thatoi et al. (eds.), *Bioprospecting of Enzymes in Industry, Healthcare and Sustainable Environment*, https://doi.org/10.1007/978-981-33-4195-1_13

13.1 Introduction

Enzymes—the indispensable constituent of bio-based chemistry are the most sought after biocatalyst in the twenty-first century. Chemical-based catalyst has been in use since time immemorial but owing to various disadvantages microbial based enzymes are in great commercial demand. Microbial enzymes are highly specific, can adjust to temperature variability, and can cause reactions at higher rates in comparison to their chemical-based catalysts (Drauz and Waldmann 2012). *Bacillus* sp. have better advantange than the other microbes as they can release extracellularly their enzymes like protease, amylase, xylanase, lipase, arabinose, etc. that are very crucial as precursor molecules (Joo et al. 2007). Industrial criteria for a good enzyme focus on stability, specificity, and biodegradability (Kumar et al. 2012). Microbial enzymes are used in almost every industry like food and beverages, textile, paper, biofuel, saccharification, etc. (Drauz and Waldmann 2012; Gupta et al. 2003). Enzymes from extreme sources like thermophiles and psychrophiles are more suitable for industrial applications.

Thermophiles are a branch of extremophiles derived from a Greek word "thermotita" which means "heat-loving organisms." Thermophiles are the microbes that usually reside above 40 °C (Horiike et al. 2009). Thermophiles can be distributed into three types depending on their optimum range of temperature—(1) moderate thermophiles have optimum growth temperature around 40 °C–60 °C (e.g., *Clostridium tepidiprofundi*); (2) true thermophiles have optimum growth temperature in the range of 60 °C–70 °C (e.g., *Thermus aquaticus*) and (3) hyperthermophiles have optimum growth temperature above 80 °C (e.g., *Thermus flavus*) (Pikuta et al. 2007). Thermophilic microorganisms have acknowledged a great curiosity in modern days because their proteins and enzymes are not usually denatured at high temperature. Most of the mesophilic enzymes get denatured at around 40 °C and are completely inactivated beyond 60 °C, whereas some thermophilic enzymes show a greater activity at 80 °C and above as well as a greater half-life at these temperatures (Satyanarayana et al. 2005).

Thermophiles are naturally designed in such a way that they can tolerate the extreme environments in which they inhabit. An increase in temperature changes their membrane and protein dynamics to withstand the environmental extreme conditions (Pikuta et al. 2007; Satyanarayana et al. 2005; Tattersall et al. 2012).

A psychrophilic prokaryote is characterized by those organisms which grow optimally below 15 °C temperature, maximum growth at 20 °C, with no growth above 20 °C temperature (Canganella and Wiegel 2011) and thus they can survive cold environments (Dalmaso et al. 2015; Margesin and Miteva 2011). Another term is "psychrotolerant" which optimally grows above 20 °C and can tolerate less than 5 °C (Canganella and Wiegel 2011). True psychrophiles are called "stenopsychrophiles" (Dalmaso et al. 2015). Cold environments include permafrost, arctic, and Antarctic ice, rocks in very cold regions, permanent cold seawater of polar regions, permanently cold marine (−2 °C), freshwater, deep rock aquifers, and all cold-blooded organisms (Canganella and Wiegel 2011; Miteva and Brenchley 2005; Miteva et al. 2004).

Adaptation of the organism at these low temperatures is due to cold shock proteins and RNA chaperons (Dalmaso et al. 2015). These cold shock or cold adaptive proteins first binds with RNA molecule to maintain its single-stranded (ss) conformation and these cold shock domains (Cold shock protein + RNA) then facilitate cold adaptation (Ramana et al. 2000). Other factors involved are secondary cold-active metabolites, enzymes, antifreeze protein and more important membrane fluidity (Casanueva et al. 2010). The higher content of alpha-helix in protein leads to maintain flexibility at low temperature (Madigan et al. 2009). The high content of unsaturated fatty acid in lipid helps to maintain semifluid state in the membrane to adapt in these extreme temperatures (Deming 2009). Less side-chain interaction among proteins also allows enzymes to be functional at minimal kinetic energy (Satyanarayana et al. 2005).

13.2 Hot Springs of Sikkim

The natural groundwater discharge point through which elevated temperate water flows to its environment is called hot spring (Sen et al. 2010). Every hot spring has its definitive characteristics like temperature, pH, and salinity, which governs the ecological niche constitution and characterizes the microbes present in its habitat. Temperature acts as a natural selection criterion that which dictates their morphology and allows the growth of only those microbes which can withstand higher temperature or tolerate these extreme conditions which can vary from 40 °C to 120 °C or above (Kumar et al. 2013; Sharma et al. 2013). The predominant thermal ecologically active regions are geothermally heated soils, hot springs, geysers, fumaroles, and solfatara (Adiguzel et al. 2009; Sen et al. 2010).

In India, the geothermal exploration began in early 1973 by Geological Survey of India and they reported more than 350 hot springs having temperature range varying above 40 °C–100 °C throughout the entire subcontinent region. Based on the tectonic movements, the hot springs of India were categorized into orogenic and non-orogenic (Bisht et al. 2011). Sikkim naturally hosts many hot springs. It is a major tourist attractive state of India where nature is in its juvenile form and a refreshing season greets its visitors. In local languages, these Hot Springs are called as *Tatopani* or *Tsha chu*. *Tatopani* is a Nepali word where "*Tato*" means Hot and "*pani*" means water whereas "*Tsha chu*" is a Tibetan word where "*Tsha*" means Hot and "*chu*" means water. Here at Sikkim, hot springs are sociologically very significant and hold a prime importance (Das et al. 2012a). It is regarded as an elixir and is believed that bathing in it can cure many bone-related diseases and drinking it can also cure gastric problems (Das et al. 2012b). Located at various places Yumthang, Yume Samdung, Tarum, Polok, Borong, Reshi, etc. hot springs are major tourist attractions.

Polok *Tatopani* (Ralang *Tchu*/Ralang *Tsha chuu*/Rabong *Tatopani*) is located at the base of Gangyab, West Sikkim by the banks of the river Rangeet (Thakur et al. 2013). The trail to the *Tatopani* spot is from Polok, South Sikkim but the *Tatopani* and its source is located at Gangyab foothill, West Sikkim just away from the River

Rangit. **Borong *Tatopani*** is located at lower Borong and the ponds are situated at the banks of river Rangit, in West Sikkim. There are three ponds for bathing but it depends on seasons. **Reshi *Tatopani*/***Phur Tsha Chu* ("*Phur*" means bubble in Tibetan language) and is located approximately 25 km from Jorethang to the east of Reshi (Tinkitam) (Sherpa et al. 2013). Hot spring source is located near the bank of river Testa. Separate time table for male and female hot spring goers were provided by *Phur Tsha Chu* committee (5–8 am for men, 8–11 men, 11–2 pm women, and after 2 pm onwards till 5 pm for men). One can feel a strong sulfurous smell from going closer to the hot spring vicinity. **Yumthang *Tatopani*** is located on the base of the mountain across the river Lachung Chu in the town of Lachung. **Dzongu *Tatopani*** is located in the valley of Lower Dzongu, Sikkim. Dzongu is closely associated with three terms—Land of Lepchas, natural hub of medicinal plants, and interaction of nature and culture. A huge pipe has been connected to this bore channel and it was connected to the bathing house where artificially two ponds have been constructed. Ponds are like modern pools for bathing purposes. Two separate bathing ponds are present—one for males and the other for females in separate two rooms. The water is used only for the bathing purpose (Das et al. 2016). **Yume Samdung *Tatopani*** is located in the North Sikkim district at Yume Samdung valley. It is above zero point and is located at the highest altitude. **Takrum *Tatopani*** is located at Lachen valley in the North Sikkim district.

13.3 Glaciers of Sikkim

Glaciers are the delicate and susceptible biomarkers used for climate change estimation. Biotic and abiotic factors in nature can influence their response like glacial length, temperature, glacier mass balance, and snowline. These factors directly or indirectly affluence the climatic response in the ecological niche (Agren 2010). Himalayan glacier retreat has been in reports since 1850 or "Little Ice Age" which led to an increase in atmospheric temperature (Armstrong 2010; Zemp et al. 2008). They approximately are spread over 33,050 sq. km. (Zemp et al. 2008) which corresponds to ~29% glaciers of Central Asia; ~5% ice caps, and other glaciers of the world. During glacier retreat, the soil gets exposed and is succeeded by algal, fungal, and plant biome in the niche (Bajracharya et al. 2007). Chronosequence created by glaciers presents a shift in the energy and biosphere which ultimately affects the ecology and further the dwelling livelihood of habitants (Gurung and Bajracharya 2012).

In the context of Sikkim Himalayas, here the retreat of glaciers has minimal documentation as only a few have been accessible. There have been geological perspective researches on glacial forelands, moraines, snow-cover, etc. (ICIMOD 2009), in the Himalaya but microbial community analysis, and their niche study is sparse. It has been hypothesized that there might be various factors that regulate the microbial niche in the glaciers like aerosol deposits, dust particles, wind velocity, light intensity, altitude, etc. (Zhang et al. 2007). The wet/dry cycles of glaciers also control the glaciation process and this phenomenon can change the glacial flux dynamics of snow deposition and microbial accumulation or transportation. The bacterial deposits can be found within the ice core sections and they can help in deciphering the microbial response to the local weather conditions at the deposition time (Priscu et al. 2007).

Sikkim hosts many glaciers within its vast geography—Zemu Glacier, Tasha Glacier, Talung Glacier, Jumthul Glacier, Lhonak Galcier, Rathong Glacier, Theukang Glacier, Teesta Glacier, Tenbawa Galcier, Tongshong Glacier, Chuma Glacier, Umaram Glacier, Changsang Glacier, Yulhe Glacier, etc. (Sherpa 2018). But among all of these only two glaciers, Rathong and Zemu have been studied both geologically and microbiologically. These are also of prime importance as they are the source of Rangit and Teesta rivers. Sikkim Himalayas have recently been subjected to high seismic shocks. The earthquakes, cyclones, avalanches, precipitation, etc., cause an impact on the glaciers and may play a role in the rapid melting of glaciers.

Our research work was concentrated on four glaciers of Sikkim—Changme Khangpu (CK), Changme Khang (CKG), Chumbu, and Kanchengayao glaciers. They were selected as they had not been documented ever and had no reports of any study earlier. They were virgin glaciers in terms of their research and glaciological studies. CK and CKG glacier are situated at Sebu valley of Teesta river basin in North Sikkim (Sherpa et al. 2018). CKG glacier is debris-free glacier and CK is a debris cover glacier. Chumbu glacier originated from the south slope of Chumbu peak. Meltwater of these glaciers feeds into Sebu basin which is ultimately merged into Lachung river of North Sikkim. Kanchengayao glacier, on the other hand, is located at Lachen, Thangu valley, North Sikkim, India. Kanchengayao glacier is a debris-free transverse valley glacier. Kanchengayao glacier originated from south slope Mt. Kanchengayao peak, trending north-south face. Meltwater of this feeds Thangu River of Lachen River, Lachen, North Sikkim.

13.4 Biotechnological and Industrial Significance of Extremozymes

13.4.1 Thermophiles and Thermozymes

Biotechnology has clearly changed our lives in many captivating ways which are inexorable. Many of the reactions involved in biotechnological or industrial processes to develop outputs, need to take place on extremes of temperature, pH, pressure, and salinity (Coker 2016). The mesophilic macromolecules can be utilized in these processes, but being temperature susceptible, these macromolecules must be genetically or chemically modified to harvest the products. However, these modifications can be lengthy and cost-effective (Siddiqui et al. 2009). In contrast, nature has fervently provided with suitable alternatives in the form of extremozymes which are present in microorganisms that can bloom in extremes of temperature, pressure, salinity, and pH (Deming and Baross 2001). These naturally thermostable macromolecules are being already used in various industrial processes. Nonetheless, the chase has additionally filled in the previous quite a while by industry's acknowledgment that the "survival units" controlled by extremophiles can conceivably serve in a variety of uses. The various applications which have made paradigm shifts in the field of biotechnology are the discovery of polymerase from a thermophilic bacterium *Thermus aquaticus* (Ishino and Ishino 2014). Other applications include biofuel production using various thermophilic enzymes (Barnard et al. 2010), thermophilic microorganism used in biomining (Johnson 2014) and carotenoids used in the food and cosmetic industries (Oren 2010).

Enzymes obtained from thermophilic microorganisms have incomparable physiognomies, for example, temperature, pH, and chemical stability. These proteins or enzymes are inherently more stable under extreme environments than those present in their mesophilic analogs (Satyanarayana et al. 2005). Thermal sensitivity has been the foremost problem to the widespread use of enzymes as far as industry is concerned. The benefits of thermozymes are that due to high temperature there is a lesser chance of contamination, improved reaction rates, substrate solubility, and lower viscosity (Joshi and Satyanarayana 2013). From the biotechnological point of view, the thermophiles are the most attractive microbes on earth due to their ability to produce enzymes that can easily catalyze industrial processes at higher temperatures than their corresponding mesophiles. Thus, the ability of thermophilic enzymes to suitably work at high temperature implies many advantages for their applications in industry (Satyanarayana et al. 2005). Thus, these thermostable enzymes held an explicit allure for researchers all over the world. Mesophilic hosts like *E. coli*, *B. subtilis,* and yeasts were successfully cloned by the thermo enzyme encoding genes for retrieving archaeal genes (Deming and Baross 2001).

13.4.1.1 DNA Polymerases

The discovery of natural stability of DNA polymerase at higher temperature led to the introduction of robust PCR method. PCR is generally used to amplify the nucleic acid sequences, which in turn has found several applications directly or indirectly in biotechnology, genetic engineering, medical, pharmaceutical, and many other fields. Besides, PCR, DNA polymerase enzyme is exploited in DNA cloning, DNA sequencing, whole genome amplification (WGA), single nucleotide polymorphism (SNP) detection, molecular diagnostics, and synthetic biology (Gardner and Kelman 2014). There are many steadfast DNA polymerases used in the above techniques such as *Taq*, *Pfu,* and *Vent* which were isolated from thermophiles *Thermus aquaticus*, *Pyrococcus furiosus*, and *Thermococcus litoralis,* respectively (Satyanarayana et al. 2005). *Taq* polymerase was industrialized and $2 billion royalty was earned by PCR rights holders during its patent (Fore et al. 2006). The distinguishing characteristics of each DNA polymerase may encourage the impending advancement of exclusive reagents and thus the exploration of a new type of DNA polymerase will be of prime foremost emphases in future studies (Ishino and Ishino 2014).

13.4.1.2 Biofuel Production

Alternative support to replenish the nonrenewable resources such as fossil fuels, there is a strenuous effort to produce analogous fuels using biomassess such as corn, sugar cane, and wheat etc. These are known as the biofuels (Coker 2016). The classification of the biofuels can be carried out based on the consumption of source and on the basis of product formed. Thus, based on the source utilization, biofuels are known as first-generation biofuels which can be derived from easily hydrolyzing sugars like starches or oils; or second-generation biofuels which are generated from not easily hydrolyzed such as lignocellulosic material. However, on the basis of end product, biofuels can be classified as bioethanol, biodiesel, bio-butanol, hydrogen, and methane (Luque et al. 2008). It is known that various stages in biofuel production encompass extremes of many physical conditions such as temperature and pH. Thus, thermophiles are the ideal entrants to replace their mesophilic analogues (Coker 2016). The other benefits using thermophilic microorganisms is that the thermophiles can easily ferment sugars from biomass or even complex carbohydrates and thus can be easily exploited in the production of second-generation biofuels (Sommer et al. 2004). Moreover, thermophilic fermentations are less disposed to other microbial contaminations. Also, product inhibition is reduced as the volatile products can be easily removed (Barnard et al. 2010).

Various thermophilic and hyperthermophilic microorganisms have been exploited in biofuel production. Although the earlier traditional methods of biofuel production such as bio-butanol and bioethanol incorporate the use of chemical processes complemented with mesophilic microbes such as *Saccharomyces cerevisiae* and *Clostridium* species (Lee et al. 2008). Other thermophiles such as thermophilic *Clostridia* (fermentative anaerobes) can degrade lignin-containing substances. They possess a multienzyme complex called cellulosome in their cell membranes. This cellulosome complex has the potential to ensure the enzymatic degradation of cellulosic substances (Demain et al. 2005). *Geobacillus* are the other promising thermophilic candidates for ethanol production. Certain species of *Geobacillus* can degrade complex carbohydrates such as xylan due to the production of xylanase enzymes by them (Wu et al. 2006). *Geobacillus stearothermophilus* produces ethanol at higher temperatures and with a good yield similar to that of *S. cerevisiae* (Bibi et al. 2014). Other species of *Geobacillus* can tolerate 10% ethanol concentration like *Geobacillus thermoglucosidasius* (Fong et al. 2006). Thus, thermophilic microorganisms have produced a great amount of interest in biofuel production.

13.4.1.3 Bioremediation and Biomining

Substantial metal contamination speaks to an essential issue because of its dangerous impact and aggregation all through the natural pecking order which prompts genuine environmental and medical issues (Najar 2018). From environmental perspectives, the removal and recovery of heavy metals are very important (Nourbakhsh et al. 2002). Several reports on eubacteria and fungi are available which deals with metal tolerance (Cánovas et al. 2003). However, hyperthermophilic bacteria were also established for bioremediation of heavy metals at higher temperatures (Rajendran et al. 2003; Sar et al. 2013). It has also been known that there is active (bioaccumulation) and passive (adsorption) uptake of heavy metals by microorganisms (Hussein et al. 2004). A thermophilic bacterium *Anoxybacillus flavithermus* possess metal binding capacity showing less affinity toward Cu than Mn was enhanced through forming metallo-ligand complexes with -COOH, -PO_3, and –OH moieties (Burnett et al. 2007). Also, various thermophilic genera such as *Bacillus, Anoxybacillus, Brevibacillus*, and *Geobacillus* were investigated for sensitivity and adsorption of Cd (Hetzer et al. 2006). Temperature, pH, inoculum metal concentration, contact time, and biomass concentration were found to be the main conditions for equilibrium adsorption of Zn, Mn, Cd, Ni, and Cu for *G. thermoleovorans* sub species *stromboliensis* and *G. toebii* sub species *decanicus* (Özdemir et al. 2009).

Biomining generally called bioleaching, is the amputation of insoluble metal sulfides or oxides by using microorganisms (Donati et al. 2016). It has been estimated that the extraction rates using biomining are around 90% compared with 60% for traditional heap leaching (Vera et al. 2013). Biomining has been potentially exploited in the mining of various metals such as gold, copper, silver, nickel, zinc, and uranium (Donati et al. 2016). The use of thermophiles has various advantages for biomining as compared to their mesophilic counterparts. The exploitation of thermophiles reduces the possibilities of acid mine drainage (AMD) which is sometimes caused by mesophiles is the acidic water, created by the oxidation of sulfides from the mine, starts streaming or filtering out of the mine and is cost-effective (Sheoran et al. 2010). Many thermophilic strains, such as *Sulfolobus* and *Metallosphaera* have also been employed in biomining (Vera et al. 2013). Thus, the above studies suggest that the thermophilic bacteria and archaea are suitable candidates that can be potentially exploited in bioremediation and biomining (Deming and Baross 2001).

13.4.1.4 Starch Hydrolyzing Enzymes

Starch is one of the most important carbohydrate polymer made of two fractions amylopectin and amylose (Drauz and Waldmann 2012). Amylopectin molecule is a highly branched polysaccharide composed of D-Glucose residues linked with alpha 1–4 linkages and the branches are linked by α (1–6) linkages whereas the amylose molecule is a linear polysaccharide composed of D-Glucose residues linked with alpha 1–4 linkages (Robyt 2008). The degree of polymerization and the comparative content of the monomers depend on starch sources (Drauz and Waldmann 2012). Depolymerization or degradation of starch is carried out by enzymes known as amylases. Amylases are synthesized by animals, plants, and microorganisms and classified as alpha (α), beta (β) and gamma (γ) amylases. Alpha (α) amylases are endo-acting enzymes leading to the hydrolysis of α (1–4) linkages randomly and are unable to break α (1–6) linkages, thus α-amylases can lead to the formation of linear, branched oligosaccharides and limit dextrins. Beta (β) amylases are exo-acting and leading to the hydrolysis of only α (1–4) linkages. These acts on the polysaccharide chain from their nonreducing end, resulting in the formation of major oligosaccharide maltose. Gamma (γ) amylases are exo-acting and attack the substrate from nonreducing ends which leads to hydrolysis of both α (1–4) and α (1–6) linkages thus results in the formation of monosaccharides as a major product (Abd-Elhalem et al. 2015; Horváthová et al. 2001; Sen et al. 2014). The amylases can be exploited through wide options or applications like fermentation, textile, food, detergent, pharmaceutical, etc. (de Souza and de Oliveira 2010). Since the hydrolysis of starch related to industrial progressions requires high temperature and pH. Thus, thermophilic amylases would be the first choice for industrial purposes and thus it is not surprising that thermophilic amylases have several applications in industrial microbiology (Coker 2016). Many thermophilic microbes have shown significant amylolytic activity such as *Sulfolobus acidocaldarius*, *Sulfolobus solfataricus*, *Thermophilum, Desulfurococcus, Thermococcus,* and *Thermotoga*. It was shown that *Thermotoga maritima* a thermophilic bacterium possesses all the three amylolytic properties i.e., α, β, and γ amylase activity. Also, *Pyrococcus furiosus* and *Pyrococcus woesei,* hyperthermophilic bacteria were reported to possess highly thermostable amylolytic activities (Najar 2018).

13.4.1.5 Proteases

Proteases are the class of enzyme that converts the protein into amino acid and peptides. They are classified according to the nature of their catalytic activity (Ellaiah et al. 2002). Today the quantity of commercialized protease production in the world is as large as compared to the other biotechnologically modified enzymes. In leather, pharmaceutical, food, and textile industry, these are the major used enzymes (Li et al. 2012). Serine alkaline protease is used in addition to detergents for laundering. The proteases that can catalyze responses under outrageous condition i.e., high temperature and extraordinary pH are profitable for modern applications (Drauz and Waldmann 2012). Extremophilic proteases usually are serine rich structure which renders them thermostable at high temperature despite in presence of detergents (Ellaiah et al. 2002). Many studies have been done on thermophilic bacteria and archaea to get the promising proteolytic enzymes. A hyperthermophilic archaeon *Thermococcus kodakarensis* KOD1 was studied and a highly heat-stable protease enzyme Tk-subtilisin has been isolated. It has also been shown that after recombination, this enzyme shows optimal activity at 100 °C and was readily stable under high concentrations of various denaturants (Koga et al. 2014). Another thermopsin like protease SsMTP-1, thermostable, and pH was isolated from *Sulfolobus solfataricus,* a thermophilic archaeon (Gogliettino et al. 2014). The thermophilic bacteria have also been exploited such as a thermophilic bacterium *Coprothermobacter proteolyticus* (Toplak et al. 2013). From this bacterium proteolysin (serine protease) was isolated and is an excellent candidate in the detergent industry due to its extreme stability at high temperature and elevated pH ranges.

13.4.1.6 Lipases

Lipases hydrolyses the amalgamation of esters from glycerol and long-chain unsaturated fats. They are viewed as the most flexible proteins of the enterprises which achieve a scope of bioconversion response (Sharma et al. 2011), which incorporates hydrolysis, inter esterification, esterification, and acidolysis (Andualema and Gessesse 2012). The esters produced by lipase catalysis are crucial in the food and beverage industry for enhancing the taste and aroma (Najar 2018). Other products formed from lipase activity such as long-chain CH_3- and C_2H_4- esters of COO- moieties are used as diesel engine fuels (Jeong and Park 2008). On the other hand, ester of long-chain COO- and -OH moieties are used as additives or lubricants in cosmetics (Andualema and Gessesse 2012). The lipases have been also exploited in various other applications such as in the paper industry, casein hydrolysis in the dairy industry, non-cellulosic impurities removal from pre-processed raw cotton, drug formulations in pharmaceuticals industries, and subcutaneous fat removal in the leather industries (Andualema and Gessesse 2012). Lipases extensively produced by various microbial communities like bacteria, fungi, and yeast (Sharma et al. 2011). There are many reports on the *Bacillus* sp., as the major contributor and producer of lipolytic enzymes. In order to tolerate the extreme conditions of temperature or pH, the hunt for thermophilic lipolytic enzymes was carried out. The isolation of two thermostable and alkaline lipolytic enzymes was purified from two thermostable archaea *Sulfolobus acidophilus and Pyrobaculum* sp. (Shao et al. 2014; Zhang et al. 2014). Other thermophilic species were also being exploited for lipolytic enzymes such as *Thermotoga maritima* and *Thermus thermophilus* (Wei et al. 2013). It has also been shown that the thermophilic *bacillus* produces lipase enzymes with greater activities and stabilities than their mesophilic analogs. Thus, these stabilities of thermophilic bacteria and their enzymes signify exceptional entrants for industrial applications.

13.4.1.7 Other Enzymes

There are various other enzymes studied from thermophilic bacteria and archaea such as cellulases, esterases, pullulanases, dehydrogenases, pectinases, chitinases, isomerases, xylanases and DNA-modifying enzymes. Cellulose is the most abundant polymer on earth can be hydrolyzed by complex enzymes known as cellulases. Cellulase enzymes include endoglucanase, exoglucanase, and β-glucosidase (Acharya and Chaudhary 2012). Cellulases are important catalysts in various industrial applications such as food, detergent, textile, pulp, and paper. These cellulases can also be exploited in ethanol production (Kuhad et al. 2011). Various thermophilic bacteria have been studied such as an anaerobic thermophile *Clostridium thermocellum* has shown cellulolytic activities. Thermophilic *Bacillus* isolated from hot springs (India) also has shown cellulolytic activity (Acharya and Chaudhary 2012). Other highly thermostable cellulases stable at temperatures between 95 and 115 °C has been isolated from *Thermotoga maritima* MSB8 and *Thermotoga* sp. FjSS3-B1 (Najar 2018). Similarly, other enzyme such as esterase has been studied in some thermophilic microorganisms. Esterases catalyze the hydrolysis of ester bonds (Bornscheuer 2002). Thermo-stable acetyl xylan esterases are of great interest nowadays and have been also extracted from anaerobic microbes *Clostridium thermocellum*, *Thermoanaerobacterium* sp., etc. The phenolic acids are the precursors to many by products and can be explored in biorefineries and can also be exploited in food and cancer gene therapy (Sood et al. 2016). Besides these above-discussed applications of thermophilic microorganism and their thermo stable enzymes, there are many other fields where they can be significantly exploited in the fields of agriculture, dairy, medical, cancer treatments, etc.

13.4.2 Psychrophiles and Cryozymes

With the advancement of science and technology, the world has reached a different stage today. Working at the molecular level has led to the age of genomics and systems biology that have made possible the processes that find applications in agricultural, food, medical, and textiles industries. Moreover, due to increasing environmental concerns, more emphasis is being given upon natural, biological ways of processing and production rather than not so eco-friendly chemical means. In this aspect, enzymes are finding good applications in industries; particularly cold-adapted enzymes are of great potential for biotechnological application (Margesin et al. 2007; Miteva 2008). Much of the earth's biosphere is permanently cold (Priscu and Christner 2004). Organisms that thrive in such harsh climate, comprising of members from archaea, bacteria and eukarya, are successfully adapted to their environment (Cavicchioli 2006; Deming 2009; Margesin et al. 2007). Their adaptations enable them to grow and perform metabolic activities similar to their mesophilic and thermophilic counterparts. Hence, psychrophiles and psychrotrophs serve as natural reservoirs for enzymes that can function actively at low temperatures, and these cold-active enzymes have huge biotechnological potential (Cavicchioli et al. 2011). These cold-evolved enzymes with high catalytic efficiency are referred to as cold-active enzymes (Cavicchioli et al. 2011; Ramana et al. 2000). These are temperature sensitive on comparison with mesophiles and thermophiles. All cryozymes share one common property: thermo labile activity. The active site of the enzyme is assumed to be most heat-labile in nature. The low stability and heat-labile activity are the results of increased flexibility of the protein or its active site (Feller 2017). High flexibility to the thermo labile cold-adapted enzymes is contributed by many factors such as decreased H-bonding and other electrostatic interactions, lesser core hydrophobicity, enhanced surface hydrophobicity, longer loops with lesser proline residues, increased glycine residues, lesser disulfide bridges, etc. (Cavicchioli et al. 2011). They include protease, alpha-amylase, lipase, cellulase, esterase, xylanase, DNA ligase, alkaline phosphatase, chitinase, pectinase, alpha-lactamase, and many more (Joshi and Satyanarayana 2013). Cold-active enzymes have found huge industrial and biotechnological applications owing to high specificity at low temperature, highly thermolabile nature at increased temperature (Joshi and Satyanarayana 2013). Cryoenzymes are economical to use thereby saving energy costs. Due to their heat-labile nature, these cold-active enzymes are very useful in case of enzyme reactions where heat-sensitive substrates are being handled and it avoids undesirable by-products (Cavicchioli et al. 2011). Because of these properties, cryo-enzymes are the most useful in the food and beverage industry where maintenance of nutritional and functional value of food, their flavor and taste is very important (Joshi and Satyanarayana 2013). Cryo-proteases comprise of a significant group of enzymes as they are hydrolytic enzymes involved in most of the physiological and metabolic functions. It has been seen that at present, more than 70% of commercialized enzyme is proteases. Cryo-proteases are widely used in various fields such as food and dairy, baking, pharmaceuticals, cosmetics, textiles, leather processing, environmental bioremediation, and many more (Joshi and Satyanarayana 2013). Psychrophilic proteases have been modified to enhance their stability and catalytic activity. They seem promising for future aspects as efficient therapeutic agents (Ramana et al. 2000). Along with their wide industrial applications, cold-active enzymes are equally important in molecular sciences. Alkaline phosphatase is one of the important DNA-modifying enzymes extracted from psychrophiles. Another novel cold-adapted cellulase enzyme has also been discovered that seems very useful as at low temperatures it can convert cellulosic waste materials to biofuel (Cavicchioli et al. 2011). A wide range of psychrophiles as well as psychrotrophs produce various cold-active enzymes that can optimize the cost and efficiency of present-day industrial processes. There is scope for the development of newer techniques with lesser energy input and thereby reduction in cost by removal of cost for heat inactivation step (Joshi and Satyanarayana 2013).

13.4.2.1 Biotechnological Importance of Psychrophiles

Cryozymes have created particular interest in food and beverage industries because of their functioning at low temperatures, which will minimize the incidence of spoilage and alteration of nutritional value, taste, or quality of the product. Cold-active proteases can enhance the organoleptic property of frozen meat products (Margesin et al. 2007). Cold-active proteases, lipases, amylases, and xylanases are useful for baking in preparation of dough and processing, giving larger volumes of dough (Joshi and Satyanarayana 2013). Cryozymes have importance in pharmaceutical industries. Increasing concern for pure drugs has led to the need of biocatalysts for organic synthesis (Margesin et al. 2007). A heat-labile lipase from *Candida antarctica* has been isolated and applied broadly in modification of polysaccharide, resolution of alcohols, etc. (Margesin et al. 2007). Cryozymes have wide uses in cosmetic industries as they enhance biotransformation reactions, thus preserving the volatile substrates like fragrance (Margesin et al. 2007).

13.4.2.2 Application in Molecular Biology

Even in molecular biology, cold-active enzymes play vital reactions in sequential reactions. Alkaline phosphatase dephosphorylates vector priming prior to cloning so that self-ligation does not occur, and for removing 5’phosphate group from DNA before end-labeling (Cavicchioli et al. 2011; Margesin et al. 2007). The heat-labile nature of cryozymes is beneficial as it can be heat-inactivated after its function is over. Cold-adapted ligases can provide a better yield of ligation at low temperatures and thus is advantageous over mesophilic ligases (Margesin et al. 2007). Cold-active enzymes are further being studied for their exploitation in various biotechnological and molecular fields, to provide a convenient, economical, and cost-effective means.

13.5 Diversity and Enzymology of Thermophilic Bacterial Isolates from Hot Springs of Sikkim

The culture-dependent isolation of the thermophilic bacteria producing industrially important enzymes was done from four different hot springs of Sikkim. The culture-dependent studies showed the complete dominance of phylum Firmicutes in the hot springs of Sikkim. *Geobacillus* was the predominant genus along with few representatives of *Anoxybacillus* and *Bacillus*. Our study through culture study showed that—*G. stearothermophilus* XTR25, *G. kaustophilus* YTPR1, *G. subterraneus* 17R4, *G. lituanicus* TP11, *G. kaustophilus* YTPB1, *Parageobacillus toebii* 10PHP2, *G. toebii* strains, *Anoxybacillus caldiproteolyticus* TRB1, *Anoxybacillus gonensis* TP9, *Bacillus smithii* 17R6, *Bacillus* sp., 17R5 were the bacterial flora present respectively. A novel bacterium was also isolated from Yumthang hot spring—*Geobacillus yumthangensis* (Najar et al. 2018a, b, c).

Culture-independent analysis through metagenomics of the hot springs of Sikkim showed various phylum diversity like—Proteobacteria (~63%), Bacteroidetes (~15%), Acidobacteria (~4%), Nitrospirae (~4%) and Firmicutes (~3%) in Borong *Tatopani*; Polok *Tatopani* had Proteobacteria (~47%), Bacteroidetes (~4%), Firmicutes (~3%), Parcubacteria (~3%) and Spirochaetes (~3%); Yumthang *Tatopani* had Actinobacteria (~98%) and Proteobacteria (~2%) in majority; Reshi *Tatopani* had Proteobacteria (~76%), Actinobacteria (~23%), Firmicutes (~1%), and Cyanobacteria (0.03%).

At genus level there was a distinct variation in hot springs. The genera present in Borong *Tatopani* had *Acinetobacter* (~8%), *Flavobacterium* (~4%), *Vogesella* (~4%), *Ignavibacterium* (~3%), *Sediminibacterium* (~3%), *Thermodesulfovibrio* (~3%), and *Acidovorax* (~2%); Polok *Tatopani* had *Flavobacterium* (~3%), *Sediminibacterium* (~3%), *Pseudomonas* (~2%), *Treponema* (~2%) and *Opitutus* (~1%); Yumthang *Tatopani* had *Rhodococcus* (~98%), *E. coli* (~0.7%), *Serratia* (~0.5%), *Nocardiopsis* (~0.5%), *Brevundimons* (~0.2%) and *Acinetobacter* (~0.2%); Reshi *Tatopani* had *Pseudomonas* (~85%), *Rhodococcus* (~4%), *Dietzia* (~4%), *Arthrobacter* (~4%), *Staphylococcus* (~1%), and *Paracoccus* (~0.3%).

The diversity at species level varied significantly in all the four hot springs. Polok *Tatopani* had *Sediminibacterium goheungense, Opitutus terrae*, *Treponema caldarium, Ignavibacterium album, Desulfobulbus mediterraneus, Thermodesulfovibrio yellowstoni*, *Hydrogenobacter thermophilus, Thermoanaerobacter uzonensis, Thermoanaerobaculum aquaticum, Thermodesulfovibrio hydrogeniphilus, Thermolithobacter ferrireducens, Thermus arciformis, Thermus caliditerrae,* etc. Borong *Tatopani* had *Ignavibacterium album*, *Rheinheimera aquatic*, *Flavobacterium cheonhonense*, *Thermodesulfovibrio yellowstoni*, *Thiovirga sulfuroxydans*, *Meiothermus hypogaeus,* etc. Reshi *Tatopani* had *Microbacterium species* (~67%), *Arthrobacter phenanthrenivorans* (~3%), and *Rhodococcus erythropolis* (~2%) and Yumthang *Tatopani* had *Rhodococcus ruber* (~98%) and *Escherichia coli* (~1%), respectively. Polok and Borong *Tatopani* had lesser amount of archaeal communities. Borong *Tatopani* had *Crenarchaeota* (~1%), whereas Polok *Tatopani* had *Euryarchaeota* (~0.6%). *Desulfurococcales* and *Desulfurococcus* were the major order and genus under *Crenarchaeota* respectively whereas *Methanomicrobiales* and *Methanospirillum* were the major order and genus under *Euryarchaeota*. However, we could not find any archaeal communities in Reshi and Yumthang *Tatopani*.

Our preliminary research on α-amylase enzymatic production showed that there were few isolates that had this property. It was found that the enzyme was functional at 60 °C and AYS8 isolate showed the highest enzymatic activity of 2.6 Units min^{-1} mL^{-1} while SY10 showed the lowest activity of 2.1 Units min^{-1} mL^{-1} at 60 °C. However, enzyme production was highest at 37 °C. The isolate AYS 8 showed the highest enzyme activity of 2.76 Units min^{-1} mL^{-1} at pH 8. α-amylase enzyme produced by the isolates isolated from Yume Samdung and Yumthang hot springs of Sikkim, indicated the enzyme was thermostable from 37 °C–90 °C (optimum = 60 °C), but isolates showed two different optimum activity at pH 7 and 8. Thermo stability is one of the important characteristics for industrial applications.

13.6 Diversity and Enzymology of Psychrophilic Bacterial Isolates from Glaciers of Sikkim

Our study in the Changme Khangpu (CK) and Changme Khang (CKG) glaciers of Sikkim through culture-dependent studies showed that the bacteria population was dominated by phylum Firmicutes and belonging mostly to *Bacillus* genus (*Bacillus cereus* KY982961; *Bacillus thuringiensis* KY982962; *Bacillus safensis* MF163138, *Bacillus oceanisediminis* MF163139, *Bacillus nealsonii* MF163141, and *Brevibacillus brevis* MF191718). The second most dominant phylum was Actinobacteria and identified belonged to *Neomicrococcus lactis* MF163142, *Pseudoclavibacter terrae* MF163143, and *Brevibacterium linens* MF1631143. The bacteria identified from Kanchengayao glacier belonged to *Pseudomonas* genus with different species such as *P. fluorescens, P. reactants, P. hibiscicola, P. maltophilia, P. synxantha, P. poae, and P. azotoformans.* The identified bacteria from Chumbu glacier belonged to *Bacillus* groups such as *Bacillus wiedmannii*, *B. velezensis, B. odorifer, and B. fusiformis* (Sherpa et al. 2018).

Culture-independent studies through metagenomics of CK glacier revealed that at phylum level, Proteobacteria were (~99%) abundant followed by unidentified virus (~0.2%), Firmicutes (0.03%), Ascomycota (0.001%), and Actinobacteria (0.14%). At class level, CK had *Beta-proteobacteria* (~66%), *Gamma-proteobacteria* (~18%), *Alpha-proteobacteria* (~15%), *Bacilli* (~0.4%), *Actinobacteria* (~0.3%), *Eurotiomycetes* (0.009%), and unidentified virus (0.27%). At the genus level, CK glacier had *Delftia* (~62%), S*erratia* (~17%), *Brevundimonas* (~15%), *Massilia* (~3%), and *Bifidobacterium* (~0.02%). Species-level classification showed the dominance of *Delftia acidovorans* (~38%), *Delftia* unclassified (~25%), *Serratia marcescens* (~17%), *Brevundimonas* unclassified (~15%), and *Massilia* unclassified (~3%).

Glacier CKG metagenomics at phylum level had Proteobacteria (~99%), followed by unidentified virus (~0.2%), Firmicutes (~0.03%), Ascomycota (~0.001%) and Actinobacteria (~0.1%). At class level classification, CKG had *Beta-proteobacteria* (~52%), *Gamma-proteobacteria* (~36%), *Alpha-proteobacteria* (~11%), *Bacilli* (~0.04%), *Actinobacteria* (~0.1%), *Eurotiomycetes* (~0.01%), and unidentified virus (~0.24%). At genus level classification, CKG glacier had *Delftia* (~49%), *Serratia* (~31%), *Brevundimonas* (~11%), *Stenotrophomonas* (~3%), *Massilia* (~2%), *Commamonas* (~0.7%). Species-level classification were dominated by *Serratia marcescens* (31%) followed by *Delftia acidovorans* (~29%), *Delftia* unclassified (~20%), *Brevundimonas* unclassified (~11%) and *Stenotrophomonas maltophilia* (~3%). Highest amylase activity was showed by isolated CK13 *Bacillus safensis* (1.07 Units mL^{-1} min^{-1}) on the other hand highest protease activity were showed by isolated CKG2 *Bacillus thuringiensis* (2.24 Units mL^{-1} min^{-1}) (Sherpa et al. 2018).

13.7 Biotechnological Potential of Polyextremophilic Bacterial Isolates

Tolerating or adapting in extreme conditions varying multi parameters like pH, salinity, and temperature is a very unique biological adaptability and these special microbes are called polyextremophiles (Chela-Flores 2013; Dhakar and Pandey 2016). They might be optimally functioning in varying the acidity or alkalinity accompanying high salinity with hyper-temperate conditions (temperature > 70 °C or methane gas conditions). The polyextremophiles have recently gained lots of research momentum. Research perspectives to understand the "know-how" mechanisms for surviving the pertinent niche sturdiness and their possible biotechnological and industrial applications.

During our research, we also have encountered such resilient species from the hot springs which showed optimal growth at wide ranges of pH and temperature. Our isolates although did not prefer high salinity rather liked minimal saline conditions present without adding any NaCl in the media. There have been many reports in extremophilic bacteria isolated from harsh environments that had neutral pH "on-field" but microbiological characteristics showed that they were able to withstand a wide range of pH (1–12) during in vitro studies. There have been reports of many such bacterial isolates like *Bacillus* and *Paenibacillus* sp. from few hot springs in India which can endure varying temperature ranges (20 °C–80 °C) and wide range of pH (5–14) (Pandey et al. 2014a). *Geobacillus stearothermophilus* (GBPI-16), obtained from Soldhar hot spring, India not only survived the autoclave conditions but can grow actively at 95 °C and produce very stable amylase and lipase (Pandey et al. 2014b). Initial studies with these isolates are encouraging, however, it required more research in these kinds of polyextremophilic bacteria. The survival mechanisms might lie with the genomic structural organization and extremozymes which renders them such poly-phasic extreme properties. The enzymes from these polyextremophilic bacteria might also have poly-phasic extreme properties which may be have more biotechnological applications.

Psychrotolerant species are other exceptions which at the lowest freezing conditions again can survive such harsh conditions and produce metabolic by-products at a wide range of temperatures and pH (Dhakar and Pandey 2016). These special characteristics feature can help the food and beverage and pharmaceutical industries. In the natural habitat, these tolerating features also in the future can relate to the ecological succession when glacier retreats occur and the soil gets exposed. They also participate in various biogeochemical pathways to recycle and replenish the nutrient cycles for e.g., the carbon cycle, nitrogen cycle, and phosphorous cycle at sub-zero temperatures (Dhakar and Pandey 2016). There have been many reports of such microbes isolated from different Himalayan regions of India and their role in various ecological phenomena like biomining or bioremediation, biocontrol, or plant growth-promoting lie characteristics. A classic example is that of the most predominant micro-flora, the psychrotolerant strain of *Serratia marcescens* (Dhakar and Pandey 2016; Sherpa 2018). Even during our study, we found this bacterium and its cohabitants were *Bacillus* and *Pseudomonas*—all famous for their quorum sensing approaches.

13.8 Conclusions

The State of Sikkim is one of those hot spots in the Himalayas where both hot and cold microbial ecology exists. And this very subtle coexistence of natural habitats enables us the freedom as researchers to explore the fundamentals of life. Ecology is very sensitive in the Himalayas as they are subjected to climatic conditions.

Our study reported here focused only on a few hot springs and glaciers. Bioengineering of industrially beneficial strains is one of our prime interest and holds a significant stake. From the hot springs, the average temperature of all our sampling sites varied from 40° to 70 °C depending on the seasons. They naturally harbor many polyextremophiles which can grow in a wide range of temperatures and pH. We have also found many novel species whose genetic makeup is unique with less (G + C) content and interesting morphology. Most of our bacterial isolates are *Geobacillus* and these are pretty exciting microbes to explore in the near future. As some of the isolates are good amylase and protease producers, this very potential has to be engineered further to step up the enzyme production and clone the genes. Thus, exploration has just begun and many more steps are yet to be taken.

Acknowledgements SD acknowledges Department of Science and Technology, Govt. of India for providing DST INSPIRE fellowship (IF130091). The authors would like to thank Forest Department, Govt. of Sikkim for their research permission and the people of Sikkim for their overwhelming support during field. Authors are grateful for the support of various funding agencies—Department of Science and Technology; Department of Biotechnology, Govt. of India for providing funds to successfully conduct researches on hot springs and glaciers of Sikkim.

References

Abd-Elhalem BT, El-Sawy M, Gamal RF et al (2015) Production of amylases from *Bacillus amyloliquefaciens* under submerged fermentation using some agro-industrial by-products. Ann Agric Sci 60:193–202. https://doi.org/10.1016/j.aoas.2015.06.001

Acharya S, Chaudhary A (2012) Bioprospecting thermophiles for cellulase production: a review. Braz J Microbiol 43(3):844–856. https://doi.org/10.1590/S1517-83822012000300001

Adiguzel A, Ozkan H, Baris O et al (2009) Identification and characterization of thermophilic bacteria isolated from hot springs in Turkey. J Microbiol Methods 79(3):321–328. https://doi.org/10.1016/j.mimet.2009.09.026

Agren GI (2010) Microbial mitigation. Nat Geosci 3:303–304. https://doi.org/10.1038/ngeo857

Andualema B, Gessesse A (2012) Microbial lipases and their industrial applications: review. Biotechnol 11:100–118. https://doi.org/10.3923/biotech.2012.100.118

Armstrong RL (2010) The glaciers of the Hindu Kush-Himalayan region: a summary of the science regarding glacier melt/retreat in the Himalayan, Hindu Kush, Karakoram, Pamir, and Tien Shan mountain ranges. ICIMOD, Kathmandu. https://lib.icimod.org/record/26917

Bajracharya SR, Mool PK, Shrestha BR (2007) Impact of climate change on Himalayan glaciers and glacial lakes: case studies on GLOF and associated hazards in Nepal and Bhutan. ICIMOD, Kathmandu. https://lib.icimod.org/record/22442

Barnard D, Casanueva A, Tuffin M et al (2010) Extremophiles in biofuel synthesis. Environ Technol 31(8–9):871–888. https://doi.org/10.1080/09593331003710236

Bibi Z, Ansari A, Zohra RR et al (2014) Production of xylan degrading endo-1, 4-β-xylanase from thermophilic *Geobacillus stearothermophilus* KIBGE-IB29. J Radiat Res Appl Sci 7(4):478–485. https://doi.org/10.1016/j.jrras.2014.08.001

Bisht SS, Das NN, Tripathy NK (2011) Indian hot water springs: a bird's eye view. J Energy Environ Carbon Credits 1(1):1–15

Bornscheuer UT (2002) Microbial carboxyl esterases: classification, properties and application in biocatalysis. FEMS Microbiol Rev 26(1):73–81. https://doi.org/10.1111/j.1574-6976.2002.tb00599.x

Burnett PGG, Handley K, Peak D et al (2007) Divalent metal adsorption by the thermophile *Anoxybacillus flavithermus* in single and multi-metal systems. Chem Geol 244:493–506

Canganella F, Wiegel J (2011) Extremophiles: from abyssal terrestrial ecosystems and possibly beyond. Naturwissenschaften 98(4):253–279. https://doi.org/10.1007/s00114-011-0775-2

Cánovas D, Durán C, Rodríguez N et al (2003) Testing the limits of biological tolerance to arsenic in a fungus isolated from the river Tinto. Environ Microbiol 5(2):133–138. https://doi.org/10.1046/j.1462-2920.2003.00386.x

Casanueva A, Tuffin M, Cary C et al (2010) Molecular adaptations to psychrophily. The impact of omic technologies. Trends Microbiol 18(8):374–381. https://doi.org/10.1016/j.tim.2010.05.002

Cavicchioli R (2006) Cold adapted archaea. Nat Rev Microbiol 4(5):331–343. https://doi.org/10.1038/nrmicro1390

Cavicchioli R, Charlton T, Ertan H et al (2011) Biotechnological uses of enzymes from psychrophiles. J Microbial Biotechnol 4(4):449–460. https://doi.org/10.1111/j.1751-7915.2011.00258.x

Chela-Flores J (2013) Polyextremophiles: summary and conclusions. In: Seckbach J, Oren A, Stan-Lotter H (eds) Polyextremophiles: life under multiple forms of stress. Cellular origin, life in extreme habitats and astrobiology. Springer, Dordrecht. https://doi.org/10.1007/978-94-007-6488-0

Coker JA (2016) Extremophiles and biotechnology: current uses and prospects. F1000 Res 5:F1000 faculty Rev-396. https://doi.org/10.12688/f1000research.7432.1

Dalmaso GZL, Ferreira D, Vermelho AB (2015) Marine extremophiles; a source of hydrolases for biotechnological application. Mar Drugs 13(4):1925–1965. https://doi.org/10.3390/md13041925

Das S, Sherpa MT, Sachdeva S et al (2012a) Hot springs of Sikkim (Tatopani): a socio medical conjuncture which amalgamates religion, faith, traditional belief and tourism. Asian Acad Res J Soc Sci Humanities 1(4):80–93

Das S, Sherpa MT, Thakur N (2012b) Sikkim's *Tatopani*—a balneotherapeutic prospect for community health in north East India. Int J Agric Food Sci Technol 3(2):149–152

Das S, Najar IN, Sherpa MT et al (2016) Biotechnological and sociological importance of hot springs of Sikkim. In: Bag N, Murugan R, Bag A (eds) Biotechnology in India: initiatives and accomplishments. New India Publishing Agency, New Delhi, pp 149–181

de Souza PM, de Oliveira MP (2010) Application of microbial α-amylase in industry—a review. Braz J Microbiol 41(4):850–861. https://doi.org/10.1590/S1517-83822010000400004

Demain AL, Newcomb M, Wu JHD (2005) Cellulase, clostridia, and ethanol. Microbiol Mol Biol Rev 69(1):124–154. https://doi.org/10.1128/MMBR.69.1.124-154.2005

Deming JW (2009) Extremophiles, cold environments. In: Schechter M (ed) The Derk encyclopedia of microbiology. Academic Press, Oxford

Deming JW, Baross JA (2001) Search and discovery of microbial enzymes from thermally extreme environments in the ocean. In: Dick RP, Burns RG (eds) Enzymes in the environment. Marcel Dekker, New York, pp 327–362

Dhakar K, Pandey A (2016) Wide pH range tolerance in extremophiles: towards understanding an important phenomenon for future biotechnology. Appl Microbiol Biotechnol 100 (6):2499–2510. https://doi.org/10.1007/s00253-016-7285-2

Donati ER, Castro C, Urbieta MS (2016) Thermophilic microorganisms in biomining. World J Microbiol Biotechnol 32(11):179. https://doi.org/10.1007/s11274-016-2140-2

Drauz K, Waldmann H (2012) Enzyme catalysis in organic synthesis: a comprehensive handbook. Wiley, Hoboken, NJ. https://doi.org/10.1002/9783527618262

Ellaiah P, Srinivasulu B, Adinarayana K (2002) A review on microbial alkaline proteases. J Sci Ind Res India 61:690–704. http://hdl.handle.net/123456789/26375

Feller G (2017) Cryosphere and psychrophiles: insights into a cold origin of life? Life (Basel) 7 (2):25. https://doi.org/10.3390/life7020025

Fong JCN, Svenson CJ, Nakasugi K et al (2006) Isolation and characterization of two novel ethanol-tolerant facultative-anaerobic thermophilic bacteria strains from waste compost. Extremophiles 10(5):363–372. https://doi.org/10.1007/s00792-006-0507-2

Fore J Jr, Wiechers IR, Cook-Deegan R (2006) The effects of business practices, licensing, and intellectual property on development and dissemination of the polymerase chain reaction: case study. J Biomed Discov Collab 1:7. https://doi.org/10.1186/1747-5333-1-7

Gardner AF, Kelman Z (2014) DNA polymerases in biotechnology. Front Microbiol 5:659. https://doi.org/10.3389/fmicb.2014.00659

Gogliettino M, Riccio A, Cocca E et al (2014) A new pepstatin-insensitive thermopsin-like protease overproduced in peptide-rich cultures of *Sulfolobus solfataricus*. Int J Mol Sci 15 (2):3204–3219. https://doi.org/10.3390/ijms15023204

Gupta R, Gigras P, Mohapatra H et al (2003) Microbial α-amylases: a biotechnological perspective. Process Biochem 38(11):1599–1616. https://doi.org/10.1016/s0032-9592(03)00053-0

Gurung J, Bajracharya RM (2012) Climate change and glacial retreat in the Himalaya: implications for soil and plant development. Kath Univ J Sci Eng Technol 8(1):153–163. https://doi.org/10.3126/kuset.v8i1.6055

Hetzer A, Daughney CJ, Morgan HW (2006) Cadmium ion biosorption by the thermophilic bacteria *Geobacillus stearothermophilus* and *G. thermocatenulatus*. Appl Environ Microbiol 72 (6):4020–4027. https://doi.org/10.1128/AEM.00295-06

Horiike T, Miyata D, Hamada K et al (2009) Phylogenetic construction of 17 bacterial phyla by new method and carefully selected orthologs. Gene 429(1–2):59–64. https://doi.org/10.1016/j.gene.2008.10.006

Horváthová V, Janeček S, Sturdík E (2001) Amylolytic enzymes: molecular aspects of their properties. Gen Physiol Biophys 20(1):7–32

Hussein H, Ibrahim SF, Kandeel K et al (2004) Biosorption of heavy metals from waste water using *Pseudomonas* sp. Electron J Biotechnol 7(1):45–53. https://doi.org/10.2225/vol7-issue1-fulltext-2

ICIMOD (2009) Mountain biodiversity and climate change. ICIMOD, Kathmandu. https://lib.icimod.org/record/7973

Ishino S, Ishino Y (2014) DNA polymerases as useful reagents for biotechnology—the history of developmental research in the field. Front Microbiol 5:465. https://doi.org/10.3389/fmicb.2014.00465

Jeong GT, Park DH (2008) Lipase-catalyzed transesterification of rapeseed oil for biodiesel production with tert-butanol. Appl Biochem Biotechnol 148(1–3):131–139. https://doi.org/10.1007/s12010-007-8050-x

Johnson DB (2014) Biomining—biotechnologies for extracting and recovering metals from ores and waste materials. Curr Opin Biotechnol 30:24–31. https://doi.org/10.1016/j.copbio.2014.04.008

Joo MH, Hur SH, Han YS et al (2007) Isolation, identification, and characterization of *Bacillus* strains from the traditional Korean soybean-fermented food, Chungkookjang. J Appl Biol Chem 50(4):202–210

Joshi S, Satyanarayana T (2013) Biotechnology of cold-active proteases. Biology (Basel) 2 (2):755–783. https://doi.org/10.3390/biology2020755

Koga Y, Tanaka SI, Sakudo A et al (2014) Proteolysis of abnormal prion protein with a thermostable protease from *Thermococcus kodakarensis* KOD1. Appl Microbiol Biotechnol 98 (5):2113–2120. https://doi.org/10.1007/s00253-013-5091-7

Kuhad RC, Gupta R, Singh A (2011) Microbial cellulases and their industrial applications. Enzyme Res 2011:1–10. https://doi.org/10.4061/2011/280696

Kumar S, Karan R, Kapoor S et al (2012) Screening and isolation of halophilic bacteria producing industrially important enzymes. Braz J Microbiol 43(4):1595–1603. https://doi.org/10.1590/S-15178382201200040000044

Kumar N, Singh A, Sharma P (2013) To study the physico-chemical properties and bacteriological examination of hot spring water from Vashisht region in district Kullu of HP, India. Int Res J Environ Sci 2(8):28–31

Lee SY, Park JH, Jang SH et al (2008) Fermentative butanol production by *Clostridia*. Biotechnol Bioeng 101(2):209–228. https://doi.org/10.1002/bit.22003

Li S, Yang X, Yang S et al (2012) Technology prospecting on enzymes: application, marketing and engineering. Comput Struct Biotechnol J 2:e201209017. https://doi.org/10.5936/csbj.2012-09017

Luque R, Herrero-Davila L, Campelo JM et al (2008) Biofuels: a technological perspective. Energ Environ Sci 1:542–564. https://doi.org/10.1039/B807094F

Madigan MT, Martinko JM, Dunlap PV et al (2009) Brock biology of microbiology, 12th edn. Benjamin Cummings, San Francisco

Margesin R, Miteva V (2011) Diversity and ecology of psychrophilic microorganisms. Res Microbiol 162(3):346–361. https://doi.org/10.1016/j.resmic.2010.12.004

Margesin R, Neuner G, Storey KB (2007) Cold-loving microbes, plants, and animals-fundamental and applied aspects. Naturwissenschaften 94(2):77–99. https://doi.org/10.1007/s00114-006-0162-6

Miteva V (2008) Bacteria in snow and glacier ice. In: Margesin R, Schinner F, Marx J-C et al (eds) Psychrophiles: from biodiversity to biotechnology. Springer Verlag, Berlin, pp 31–50. https://doi.org/10.1007/978-3-540-74335-4

Miteva VI, Brenchley JE (2005) Detection and isolation of ultra small microorganisms from a 120000-year-old Greenland glacier ice core. Appl Environ Microbiol 71(12):7806–7818. https://doi.org/10.1128/AEM.71.12.7806-7818.2005

Miteva VI, Sheridan PP, Brenchley JE (2004) Phylogenetic and physiological diversity of microorganisms isolated from a deep Greenland glacier ice core. Appl Environ Microbiol 70 (1):202–213. https://doi.org/10.1128/aem.70.1.202-213.2004

Najar IN (2018) Bacterial diversity and antibiotic resistance profile of four Hot Springs of Sikkim. PhD Thesis, Sikkim University. http://hdl.handle.net/10603/252555

Najar IN, Sherpa MT, Das S et al (2018a) *Geobacillus yumthangensis* sp. nov., a thermophilic bacterium isolated from a north-east Indian hot spring. Int J Syst Evol Microbiol 68 (11):3430–3434. https://doi.org/10.1099/ijsem.0.003002

Najar IN, Sherpa MT, Das S et al (2018b) Draft genome sequence of *Geobacillus yumthangensis* AYN2 sp. nov., a denitrifying and sulfur reducing thermophilic bacterium isolated from the hot springs of Sikkim. Gene Rep 10:162–166. https://doi.org/10.1016/j.genrep.2017.12.007

Najar IN, Sherpa MT, Das S et al (2018c) Microbial ecology of two hot springs of Sikkim: Predominate population and geochemistry. Sci Total Environ 637–638:730–745. https://doi.org/10.1016/j.scitotenv.2018.05.037

Nourbakhsh MN, Kilicarslan S, Ilhan S et al (2002) Biosorption of Cr6+, Pb2+ and Cu2+ ions in industrial wastewater on *Bacillus* sp. Chem Eng J 85:351–355

Oren A (2010) Industrial and environmental applications of halophilic microorganisms. Environ Technol 31(8–9):825–834. https://doi.org/10.1080/09593330903370026

Özdemir S, Kilinc E, Poli A et al (2009) Biosorption of cd, cu, Ni, Mn and Zn from aqueous solutions by thermophilic bacteria, *Geobacillus toebii* sub. Sp. *decanicus* and *Geobacillus thermoleovorans* sub. sp. *stromboliensis*: equilibrium, kinetic and thermodynamic studies. Chem Eng J 152:195–206

Pandey A, Dhakar K, Sharma A (2014a) Thermophilic bacteria that tolerate a wide temperature and pH range colonize the Soldhar (95C) and Ringigad (80C) hot springs of Uttarakhand, India. Ann Microbiol 65(2):809–816. https://doi.org/10.1007/s13213-014-0921-0

Pandey A, Dhakar K, Sati P et al (2014b) *Geobacillus stearothermophilus* (GBPI_16): a resilient hyperthermophile isolated from an autoclave sediment sample. Proc Natl Acad Sci India Sect B Biol Sci 84(2):349–356

Pikuta EV, Hoover RB, Tang J (2007) Microbial extremophiles at the limits of life. Crit Rev Microbiol 33(3):183–209. https://doi.org/10.1080/10408410701451948

Priscu JC, Christner BC (2004) Earth's icy biosphere. In: Bull A (ed) Microbial diversity and bioprospecting. ASM Press, Washington, DC, pp 130–145

Priscu JC, Christner BC, Foreman CM et al (2007) Biological material in ice cores. In: Encyclopedia of quaternary sciences. Elsevier, Amsterdam

Rajendran P, Muthukrishnan J, Gunasekaran P (2003) Microbes in heavy metal remediation. Indian J Exp Biol 41(9):935–944

Ramana KV, Singh L, Dhaked RK (2000) Biotechnological application of psychrophiles and their habitat to low temperature. J Sci Ind Res India 59(2):87–101. http://hdl.handle.net/12345678-9/26565

Robyt JF (2008) Starch: structure, properties, chemistry, and enzymology. In: Fraser-Reid B, Tatsuta K, Thiem J (eds) Glycoscience. Springer-Verlag, Berlin. https://doi.org/10.1007/978-3-540-30429-6_35

Sar P, Kazy KS, Paul D et al (2013) Metal bioremediation by thermophilic microorganisms. In: Satyanarayana T, Littlechild J, Kawarabayasi Y (eds) Thermophilic microbes in environmental and industrial biotechnology: biotechnology of thermophiles. Springer, Dordrecht, pp 171–201. https://doi.org/10.1007/978-94-007-5899-5

Satyanarayana T, Raghukumar C, Shivaji S (2005) Extremophilic microbes: diversity and prospectives. Curr Sci 89:78–90

Sen SK, Mohapatra SK, Satpathy S et al (2010) Characterization of hot water spring source isolated clones of bacteria and their industrial applicability. Int J Chem Res 2(1):01–07. https://doi.org/10.9735/0975-3699.2.1.1-7

Sen SK, Dora TK, Das Mohapatra PK et al (2014) Thermostable alpha-amylase enzyme production from hot spring isolates *Alcaligenes faecalis* SSB17—statistical optimization. Biocatal Agric Biotechnol 3(4):218–226. https://doi.org/10.1016/j.bcab.2014.03.005

Shao H, Xu L, Yan Y (2014) Biochemical characterization of a carboxylesterase from the archaeon *Pyrobaculum* sp. 1860 and a rational explanation of its substrate specificity and thermostability. Int J Mol Sci 15(9):16885–16910. https://doi.org/10.3390/ijms150916885

Sharma D, Sharma B, Shukla AK (2011) Biotechnological approach of microbial lipase: a review. Biotechnology 10:23–40. https://doi.org/10.3923/biotech.2011.23.40

Sharma N, Vyas G, Pathania S (2013) Culturable diversity of thermophilic microorganisms found in hot springs of northern Himalayas and to explore their potential for production of industrially important enzymes. Scholars Acad J Biosci 1(5):165–178

Sheoran AS, Sheoran V, Choudhary RP (2010) Bioremediation of acid-rock drainage by sulphate-reducing prokaryotes: a review. Miner Eng 23:1073–1100. https://doi.org/10.1016/j.min-eng.2010.07.001

Sherpa MT (2018) Microbiological analysis of two glaciers of North Sikkim. PhD Thesis, Sikkim University. http://dspace.cus.ac.in/jspui/handle/1/6345

Sherpa MT, Das S, Thakur N (2013) Physico-chemical analysis of hot water springs of Sikkim—Polok Tatopani, Borong Tatopani and Reshi Tatopani. Recent Res Sci Technol 5(1):63–67. http://updatepublishing.com/journal/index.php/rrst/article/view/1010

Sherpa MT, Najar IN, Das S et al (2018) Bacterial diversity in an alpine debris-free and debris-cover accumulation zone glacier ice, North Sikkim, India. Indian J Microbiol 58(4):470–478. https://doi.org/10.1007/s12088-018-0747-8

Siddiqui KS, Parkin DM, Curmi PMG et al (2009) A novel approach for enhancing the catalytic efficiency of a protease at low temperature: reduction in substrate inhibition by chemical modification. Biotechnol Bioeng 103(4):676–686. https://doi.org/10.1002/bit.22300

Sommer P, Georgieva T, Ahring BK (2004) Potential for using thermophilic anaerobic bacteria for bioethanol production from hemicellulose. Biochem Soc Trans 32(Pt 2):283–289. https://doi.org/10.1042/bst0320283

Sood S, Sharma A, Sharma N (2016) Carboxylesterases: sources, characterization and broader applications abstract general structure of CEs mechanism of action of CEs. Insights Enzyme Res 1:1–11. https://doi.org/10.21767/2573-4466.100002

Tattersall GJ, Sinclair BJ, Withers PC et al (2012) Coping with thermal challenges: physiological adaptations to environmental temperatures. Compr Physiol 2(3):2151–2202. https://doi.org/10.1002/cphy.c110055

Thakur N, Das S, Sherpa MT et al (2013) GPS mapping and physical description of Polok, Borong and Reshi Tatopani—Hot Springs of Sikkim. J Int Acad Res Multidiscip 1(10):637–648

Toplak A, Wu B, Fusetti F et al (2013) Proteolysin, a novel highly thermostable and cosolvent-compatible protease from the thermophilic bacterium *Coprothermobacter proteolyticus*. Appl Environ Microbiol 79(18):5625–5632. https://doi.org/10.1128/AEM.01479-13

Vera M, Schippers A, Sand W (2013) Progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation—part A. Appl Microbiol Biotechnol 97(17):7529–75241. https://doi.org/10.1007/s00253-013-4954-2

Wei T, Feng S, Mao D et al (2013) Characterization of a new thermophilic and acid tolerant esterase from *Thermotoga maritima* capable of hydrolytic resolution of racemic ketoprofen ethyl ester. J Mol Catal B: Enzym 85–86:23–30. https://doi.org/10.1016/j.molcatb.2012.08.006

Wu S, Liu B, Zhang X (2006) Characterization of a recombinant thermostable xylanase from deep-sea thermophilic *Geobacillus* sp. MT-1 in East Pacific. Appl Microbiol Biotechnol 72(6):1210–1216. https://doi.org/10.1007/s00253-006-0416-4

Zemp M, Roer I, Kääb A et al (2008) Global glacier changes: facts and figures. UNEP, Geneva and World Glacier Monitoring Service (WGMS), Zurich. https://www.zora.uzh.ch/id/eprint/4173/

Zhang S, Hou S, Ma X et al (2007) Culturable bacteria in Himalayan glacial ice in response to atmospheric circulation. Biogeosciences 4:1–9. https://doi.org/10.5194/bg-4-1-2007

Zhang XY, Fan X, Qiu YJ et al (2014) Newly identified thermostable esterase from *Sulfobacillus acidophilus*: properties and performance in phthalate ester degradation. Appl Environ Microbiol 80(22):6870–6878. https://doi.org/10.1128/AEM.02072-14