Review Paper:

Unveiling the Heat-Loving Microheroes - A Deep Dive into the World of Thermophilic Bacteria: A Review

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Abstract

Thermophilic bacteria thrive in environments exceeding 70°C, from hot springs to terrestrial oil reservoirs, they possess remarkable resilience and adaptability, making them unique in the microbial world. These heat-loving microorganisms exhibit high metabolic activity, enabling them to produce thermally stable enzymes that function optimally under extreme conditions. Such enzymes are highly valued in various industries including biotechnology, pharmaceuticals and environmental management where they offer more efficient and environmentally friendly processes compared to conventional methods.

The polymerase chain reaction (PCR) technique, essential in molecular biology, utilizes Taq polymerase from the thermophilic bacterium Thermus aquaticus. Additionally, thermophilic bacteria are being explored for applications in biofuel production, waste management and the food and beverage industry. Their ability to survive in extreme conditions also makes them important for studying the limits of life, potentially offering insights into the origins of life on the Earth and the possibilities of life beyond our planet.

Keywords: Thermophiles, Thermo-enzymes, Thermo-stability, Applications.

Introduction

In the vast spectrum of temperatures conducive to life, only microorganisms have demonstrated the remarkable ability to thrive and proliferate at both extremes. The endurance of life in high-temperature environments stands as a captivating marvel of nature. A great variety of microbes survives and grows at such elevated temperatures which are termed as Thermophiles. The word "thermophile" has been derived from two Greek words "thermotita" (meaning heat) and "philia" (meaning love). They are extremophiles that thrive at high temperatures ranging from moderate thermophiles (capable of growth at temperatures between 50°C and 64°C), extreme thermophiles (between 65°C and 79°C) and hyperthermophiles (over 80°C)[69].

Thermophilic organisms are hypothesized to be the first forms of life on the Earth and therefore, they have evolutionary significance. From the perspective of evolutionary mechanisms, learning about these organisms can help us to understand the evolution of life on Earth. The possibility of life elsewhere in the solar system and the universe can also be better explored as the conditions are just harsh. These thermophiles arouse noticeable scientific interest nowadays, not only with the aim to elucidate the mystery of life at high temperatures, but also due to the huge field of biotechnological applications of the enzymes they produce or thermozymes which are able to function under industrial harsh conditions[43].

Thermophilic environments: Extreme habitats where these microorganisms can be found, include:

a. Natural habitats like volcanic areas, geothermal areas, terrestrial hot springs, deep-sea hydrothermal vents, geothermally heated oil and petroleum reserves, sun-heated soils/sediments, mud pots and deserts.
b. Man-made environments like acid mine effluents, biological wastes, waste treatment plants, self-heated compost piles, industrial processes and water heaters[52].

Hot Springs: Hot springs are formed by the surfacing of geothermally heated ground water from the Earth's crust where the water temperature is higher than the mean air temperature. They are widespread all over the world. They usually have a high mineral content including calcium to lithium and even radium[38].

Hot springs are present in many countries throughout the world. Countries that are renowned for their hot springs include Iceland, New Zealand, Chile and Japan, but there are interesting and unique hot springs at many other places as well. The Yellowstone National Park (Wyoming, USA) has one of the highest numbers of hot springs in the world. In India, there are approximately 400 geothermal springs[75] found either solitary or in groups[42,67]. The temperature of Indian hot springs ranges from 30 to 100°C; the majority of the hot springs are not volcanic in origin.

Some examples of hot-spring sites in India are Ganeshpuri (Vajreshwari), Manikaran (Himachal Pradesh), Bendru Theertha (Karnataka), Chavalpani (Mahadeo Hills of Madhya Pradesh), Surya Kund (Bihar), Phurchachu (Reshi, Sikkim), Taptapani (near Berhampur), Atri (near Bhubaneswar), Tarabalo (Nayagarh District of Orissa), Bakreshwar (West Bengal) and Tulsishyam (Gujarat)[59]. Madhya Pradesh has several hot springs located at Dhuni Pani (Amarkantak), Salbardi region (Betul district),

Chavalpani (Pachmarhi) and Anhoni (Chhindwara-Hoshangabad) having thermal discharge ranging from 30°C to 98 °C[8, 60].

Classification of Thermophiles

Microorganisms which inhabit these high temperature environments are classified into several groups depending on their optimum temperature:

1. Organisms which can survive below 45ºC, are facultative thermophiles.
2. Organisms which have an optimum growth temperature less than or equal to 45°C but can grow at temperatures greater than 45°C as well, are known as thermo tolerant.
3. Organisms with an optimum growth temperature between 45ºC and 60ºC, are moderate thermophiles.
4. Strict thermophiles are those which have optimum growth at temperatures between 60ºC and 90ºC.
5. Organisms which grow best at temperatures greater than 90ºC, are extreme thermophiles or hyperthermophiles[4].

Factors determining heat tolerance of thermophilic organisms

1. Chemical stability: Thermophilic organisms are able to grow at high temperature due to the chemical stability of their membrane lipids[32].

2. Temperature: Lipids that increase in proportion to an increase in growth temperature may be designated as ‘‘thermophilic lipids.’’ When the growth temperature increases from 45 ºC to 65°C, the diether lipids (archaeol based lipids) decrease from 80 to 20 %, while the standard caldarchaeol-based and cyclic archaeol-based lipids increase from 10 to 40 % respectively[62].

3. Membrane Fluidity: High temperature also increases the fluidity of membranes. To maintain optimal membrane fluidity, the cell must adjust the membrane composition, that is, the amount and type of lipids. So, the membrane lipids of thermophiles contain more saturated and straight-chain fatty acids than mesophiles. This allows thermophiles to grow at higher temperatures by providing the right degree of fluidity needed for membrane function[59].

4. G+ C content: rRNA and tRNA molecules of thermophilic bacteria have higher G+ C contents than mesophiles[26]. Because the GC, base pair forms more hydrogen bonds than the AT base pair, higher G+ C contents in the double-stranded stem region improve thermostability of the RNA molecules[35,55].

5. Proteins

a. Amino acids: Thermophilic proteins show higher proportion of thermophilic amino acids like introduction of proline residues, reduction of glycine residues, smaller loops in the structure, addition of disulfide bonds, an increase of hydrogen bonds and salt bridges all help in the thermostability of proteins[20, 46].

b. Chaperonins: Some assisting proteins, such as molecular chaperonins, also facilitate protein thermostability. Chaperonins (heat shock proteins) function to refold partially denatured proteins. Thus, the upper temperature limit at which many hyper thermophiles can survive is higher due to chaperonin activity than the upper temperature at which they can grow[59].

6. DNA: Positive super coiling of DNA may be an important factor stabilizing DNA to high temperatures. All hyperthermophiles produce a unique protein called reverse gyrase[21,65]. It is a signature gene in all thermophiles without any exception. This is a type I DNA topoisomerase. It has been shown to catalyze the positive supercoiling of closed circular DNA. For various reasons, in particular, positively supercoiled DNA is more resistant to thermal denaturation than negatively supercoiled DNA[21].

Thermostable DNA polymerase: Thermostable DNA polymerase is a very important enzyme for molecular biological studies such as DNA amplification and DNA sequencing by the polymerase chain reaction (PCR).

Table 1
Classification of Thermophilic Microbes[59]

Category	Temperature	Examples
Moderate thermophile	40 ºC –60°C	*Tepidibacter, Clostridium, Exiguobacterium, Caminibacter, Lebetimonas, Hydrogenimonas, Nautilia, Desulfonauticus, Sulfurivirga , Caminicella, Vulcanibacillus, Marinotoga, Caldithrix, Sulfobacillus, Acidimicrobium, Hydrogenobacter, Thermoplasma,Mahella, Thermoanaerobacter, Desulfovibrio*
Extreme thermophile	60 ºC –85°C	*Methanocaldococcus, Thermococcus, Palaeococcus, Thermovibrio, Balnearium, Methanotorris, Aeropyrum, Methanothermococcus, Thermosipho, Caloranaerobacter, Thermodesulfobacterium, Thermodesulfatator, Deferribacter, Thermosipho ,Desulfurobacterium, Persephonella, Kosmotoga, Rhodothermus , Desulfurobacterium , Acidianus, Thermovibrio, Marinithermus, Oceanithermus, Petrotoga, Vulcanithermus, Carboxydobrachium, Thermaerobacter, Thermosulfidibacter, Metallosphaera*
Hyper thermophile	>85°C	*Geogemma, Archaeoglobus, Methanopyrus, Pyrococcus, Sulfolobus, Thermoproteus, Methanothermus, Acidianus, Ignisphaera, Ignicoccus, Geoglobus*

Use of the thermostable *Taq* polymerase eliminates the need for having to add new enzyme to the PCR reaction during the thermocycling process. Most of the thermostable DNA polymerases have been isolated from *Thermus aquaticus* known as *Taq* polymerase[12]. However, *Taq* polymerase has a major drawback. It lacks 3'-5' exonuclease proofreading activity resulting in relatively low replication fidelity. Hence, DNA polymerases from other hyperthermophiles such as *Pyrococcus furiosus* which is named as *Pfu* DNA polymerase, possessing a proof reading activity, are used for high fidelity amplification in PCR[36].

Restriction Enzymes from Thermophiles: Restriction endonucleases (REases) are enzymes that recognize and cleave DNA in a sequence specific manner. The recognition site consists of a sequence of nucleotides in the DNA duplex, typically four to eight base pairs long. Most of the commercially produced REases are isolated from the mesophilic bacteria. But the disadvantage of REases from mesophilic sources is that these enzymes are usually denatured at ambient and high temperature. As temperature produces opposite effects on both enzyme activity and stability, it is therefore a key variable in any biocatalytic process. Also, mesophilic enzymes are unstable, have low reactivity, lose activity during purification and require refrigerated transport and storage.

Thermostable REases are preferred to circumvent these problems. Bacteria from different thermophilic genera e.g. *Bacillus*, *Thermus*, *Rhodothermus*, *Thermococcus*, *Sulfolobus*, *Thermotoga*, *Pyrococcus* and *Anoxybacillus* have been reported to produce thermostable REases. REases have wide applications in molecular biology which involve DNA cloning, sequencing, mapping, restriction analysis, restriction fragment length polymorphism and many more. Recently, the potential of thermostable restriction enzymes has been explored to improve techniques like pulse field gel electrophoresis and strand displacement amplification. They have also been incorporated into PCR[59].

Types of Thermostable Restriction Endonucleases: On the basis of origin of thermostable REases, these can be divided into three broad categories:

1. REases isolated from thermophiles: A number of geothermal habitats have been screened for the isolation of thermophiles and their REases. The environments typically inhabited by thermophiles include deserts, thermal vents, volcanoes and terrestrial hot springs.

2. REases from thermophiles cloned into mesophiles: Extensive use of REases in molecular biology and biotechnological applications makes high-level production of REases inevitable. The commercial incentive is even more in case of thermostable REases. Isolation and purification of thermostable REases from their native thermophilic hosts require high fermentation costs and results in low yields.

Therefore, cloning of REases from thermophiles in mesophiles is considered to be beneficial for high-level expression of REases for high yields and economical fermentation process. *E. coli* has been used as the host organism for cloning REases in most studies due to ease of transformation and expression. Moreover, purification of recombinant proteins is well established in *E. coli* than thermophiles.

Table 2
DNA Polymerases from Thermophilic Bacteria

S.N.	DNA Polymerase	Thermophile
1.	*Taq*	*Thermus aquaticus*
2.	*Pfu*	*Pyrococcus furiosus*
3.	*KOD*	*Thermococcus kodakaraensis*
4.	*Bst*	*Bacillus stearothermophilus*
5.	*Bsu*	*Bacillus subtilis*
6.	*Tth*	*Thermus thermophilus*
7.	*Pwo*	*Pyrococcus woesei*

Table 3
REases from different geothermal habitats

S.N.	Site of isolation	REase	Organism
1.	Geothermal regions of Northern Himalayas, India	*Bfl*I	*Anoxybacillus Flavithermus*[16]
		*TspM*I	*Thermus* sp.[54]
2.	Yellowstone National Park, USA	*Bcl*I	*Bacillus caldolyticus*[7]
		*Taq*52I	*Thermus* sp.[73]
3.	The Great Artesian Basin, Australia	*Fgo*I	*Fervidobacterium Gondwanense*[3]
4.	Oil-contaminated desert soil, Kuwait	*Bst*B7SI	*Bacillus stearothermophilus*[1]

Table 4
REases Cloned from Thermophiles in *E. coli*[59]

S.N.	REase	Source organism
1.	*Pho*I	*Pyrococcus horikoshii* OT3
2.	*PspG*I	*Pyrococcus* sp.
3.	*Mwo*I	*Methanobacterium wolfeii*
4.	*Bcl*I	*Bacillus caldolyticus*
5.	*BstN*I	*Bacillus stearothermophilus* N
6.	*BsoB*I	*Bacillus stearothermophilus*
7.	*Bsl*I	*Bacillus* sp.
8.	*Taq*I	*Thermus aquaticus*
9.	*Tsp45*I	*Thermus* sp. YS45
10.	*Tsp32*I	*Thermus* sp. SM32

3. Mutant engineered REases with increased thermostability: Various studies have focused on the generation of mutants which exhibit properties of more thermostable enzymes. These include generation of mutants by spontaneous mutations, chemical mutagenesis and protein engineering of restriction enzymes. However, there has been little success for producing thermostable restriction enzymes using these methods.

A method for rapidly generating thermostable enzyme variants is to introduce the gene coding for a given enzyme from a mesophilic organism into a thermophile *Bacillus stearothermophilus*. Variants that retain the enzymatic activity at higher growth temperatures of the thermophile, are then selected. A mutant of *B. stearothermophilus* was obtained by spontaneous mutation which produced three times more thermostable restriction enzyme. Similarly, protein engineering of restriction enzymes is being tried to generate thermostable variants of restriction enzymes[59].

Advantages of Thermostable Restriction Enzymes: Less secondary structures are formed in the substrate DNA due to high temperature[57]. This makes the DNA readily accessible to restriction enzymes and the cleavage is efficient. With increase in temperature, rate of reaction also increases, fewer amounts of restriction enzymes are required in case of thermostable restriction enzymes as compared to their mesophilic counterparts. The nonspecific agents like nucleases from mesophilic sources are taken care by the high temperature at which the thermostable enzymes work and the nucleases from mesophiles are inactivated. So, there are fewer chances of nonspecific cleavages by contaminants.

Thermostable enzymes are more resistant to activity loss by repeated freeze thaw operations[40]. Thermostable restriction enzymes are more resistant to proteolytic degradation. So, there is an increased purification yield due to less degradation during purification process. The need of −20°C during storage and transportation of mesophilic restriction enzymes pushes up the cost whereas thermostable ones can be stored and transported at 4°C or even ambient temperature. All these factors discussed above lead to decrease in overall cost of manufacturing, which is a key aspect in industries.

Applications

1. In Agriculture

a. Composting: Composting is a process that converts organic waste to a humus-like end product and is not only a waste treatment technique but also a recycling method as the end product can be used in agriculture as fertilizer. Microbes play a key role as degraders during the composting process; mesophilic micro flora constitute the pioneer component, while thermophilic micro flora, the climax and also the dominant component contribute significantly to the quality of compost. The microbial community changes with the change in physico-chemical conditions of compost. In composting, temperature is directly proportional to the biological activity of microbes within the composting system. The mesophilic micro flora forms the pioneer community which rapidly breaks down soluble, readily degradable compounds, resulting in production of heat which raises the temperature of compost and thus paves the way for thermophilic micro flora at or above 45°C; the latter forms the climax community of compost.

During the thermophilic phase, high temperature accelerates the breakdown of proteins, fats and complex carbohydrates like cellulose and hemicellulose. As the supply of these high-energy compounds becomes exhausted, the compost temperature gradually decreases and mesophilic microorganisms once again take over for the final phase of 'curing' or maturation of the remaining organic matter[59].

2. In Veterinary

a. In dairy processing: Thermophilic bacilli are used as hygiene indicators of processed product, within the dairy processing context. This is because of the ability of these strains to form endospores and biofilms. The thermophilic bacilli, such as *Anoxybacillus flavithermus* and *Geobacillus stearothermophilus* are an important group of contaminants in the dairy industry.

Although these bacilli are generally not pathogenic, their presence in dairy products is an indicator of poor hygiene and high numbers are unacceptable to customers. In addition, their growth may result in milk product defects caused by the production of acids or enzymes, potentially leading to off-flavors[10].

Table 5
Thermostable Restriction Enzymes[59]

Restriction enzyme	Source	Recognition sequence	Temperature (°C)
*BstI*503	*Bacillus stearothermophilus*	5' G/GATCC3'	60–65
*PspG*I	*Pyrococcus* sp.	5' /CCWGG3'	65–85
*Bsl*I	*Bacillus* sp.	5'CCNNNNN/NNGG3'	55
*TspM*1	*Thermus* sp.	5'C/CCGGG3'	75
*BfI*1	*Anoxybacillus flavithermus*	5'CCNNNNN/NNGG3'	60
*BstP*1	*Bacillus stearothermophilus*	5'G/GTNACC3'	55–65
*Tsp49*I	*Thermus* sp.	5'ACGT/3'	65
*Tsp1DS*I	*Thermus* sp.	5'ACGT/3'	65
*Tsp1WAM 8A*I	*Thermus* sp.	5'ACGT/3'	65
*Sua*I	*Sulfolobus acidocaldarius*	5'GG/CC3'	70
*Bse*1I	*Geobacillus stearothermophilus*	5'ACTGGN/N3'	60

b. Production of Feather Meal from Feather: Feathers, waste product of poultry processing industries, are being generated in billions of tons every year[9,29,51,66]. These feathers are generally land filled or burnt which cause environmental problems[66]. Feathers are made up of >90% protein and are rich in hydrophobic amino acids and essential amino acids like cysteine, arginine and threonine[14]. Nowadays, feathers are converted into feather meal and used as poultry feed, cattle feed, fish feed etc.[9] Most popular method of conversion of feathers is by hydrothermal process where feather is cooked under high pressure at high temperature[29,51]. Hydrothermal treatments result in the destruction of essential amino acids like methionine, lysine, tyrosine and tryptophan and produced feather meal has a poor digestibility and low nutritional value[53, 72]. In the last decade, bioconversion of feather into feather meal using feather degraders has gained importance.

Biodegradation of feather can be achieved either by crude culture filtrate containing keratinases or by cultivation of keratin-degrading microorganism obtained from thermophilic bacteria[29]. Feather degradation was demonstrated mainly during fermentation[9] and a variety of cultures have been used. Among them, *Bacillus* sp. were mainly used for feather degradation[17,68] while mixed thermophilic actinomycetes[66], *Vibrio*[28] and *Streptomyces* AB1[31] were also used.

c. Keratinase as Feed Supplement: Keratinases can be directly used as additive in animal feed and can improve the digestibility and nutritional value of feed proteins. Use of crude keratinases in feed increased the amino acid digestibility of raw feather and commercial feather meal[37, 49]. Keratinases supplemented in chicken's diet improved the growth performance of broiler chickens at different ages and also the meat yield[50, 71]. Keratinase from *Bacillus licheniformis* PWD-1 is a well-studied feed enzyme and named as "Versazyme" which is approved keratinase-based feed additive. The addition of "Versazyme" in mashed and pelleted diets showed beneficial effect on early growth and feed utilization of broilers. Versazyme increased the body weight of broilers in pelleted diet[63].

3. In Medicine

a. As an immunomodulator and anti viral: Researchers evaluated the immunomodulatory and antiviral effects of an extracellular polysaccharide (EPS-2), produced by a strain of *Geobacillus thermodenitrificans* isolated from a shallow marine vent of Vulcano Island in Italy[5].

b. In Cosmetics: Keratinases from thermophilic bacteria like *Bacillus cytotoxicus* and *Bacillus licheniformis* have also been utilized in the elimination of keratin in acne and psoriasis, elimination of human callus and degradation of keratinized skin[24,27]. It might also act to remove scar and regenerate epithelia[11].

c. Drug Delivery: Keratinases are gaining importance as enhancer to increase drug delivery through nail for the treatment of onychomycosis and psoriasis, common fungal infections of nails. Keratinases can act on nail plates[23,27] to increase its porosity which may facilitate drug diffusion through nail plates.

d. Source of Exopolysaccharides: A lot of marine bacteria produce exopolysaccharides (EPS) for growth to adhere to solid surfaces and for surviving in the extreme conditions. Many such thermophilic bacteria such as *Vibrio, Alteromonas* and *Pseudo alteromonas* strains biosynthesize structurally diverse exopolysaccharides (EPS) and excrete them into their surrounding environment. The EPS functional features have found many applications in industries such as cosmetics and pharmaceutics. In particular, some EPS produced by thermophilic marine bacteria are composed of uronic acids, neutral sugars and *N*-acetylhexosamines and may also bear some functional sulfate groups. It is used in osteoarthritis treatment, in ophthalmology and in wound healing as well as in the cosmetic industry due to its visco-elastic properties and biological properties on the cartilage and skin[34].

4. In Pharmaceuticals

a. Keratin degradation: A novel thermophilic bacterium, *Fervidobacterium pennavorans*, belonging to the Thermotogales order, isolated from hot springs of Azores

Island, grows optimally at 70°C and pH 6.5. It is the first known thermophile that is able to degrade native feathers at high temperatures. With the help of these enzymes, feathers could be converted to defined products such as the rare amino acids, serine, cysteine and proline[22].

5. In Industry

a. Leather Industry: Keratinolytic enzymes are extensively used in leather industry mainly for dehairing process. Utility of sulfide for tanning process can be replaced by the use of keratinases[24, 2, 41, 70]. In this respect, many keratinases from *Bacillus halodurans* PPKS2[56], *Bacillus halodurans* JB99[61] and *Bacillus cereus* MCM-B-326[47] were demonstrated for dehairing of goat and buffalo skin.

b. Electricity generation: Studies have revealed that thermophilic electrode reducing bacteria can be used as catalyst in microbial fuel cells. The best source of thermophilic electrode reducing bacteria was found in the marine sediments of the temperate environments. It was found that sediment fuel cells were capable of producing higher electric currents at 60˚C than at 22˚C, showing that thermophilic bacteria has a greater capability to produce electricity. The advantage of using such bacteria as catalyst in fuel cells is that they have higher rates of metabolic activity which results in more electricity and they will also be more stable under the extreme conditions normally seen in industries. The most common species were the *Therminocola sp.* Because of the production of higher electrical current by the thermophiles and also because of the omnipresence of the thermophiles, they have a promising future in the application of microbial fuel cells at high temperature[45].

c. Biofuel: At present, because of the increasing cost of oil and also due to the need to reduce the emission of the green house gases and to improve the quality of air, alternate sources of biofuels from renewable sources like biogas, bioethanol and biodiesel are being examined. Bioethanol can be formed from sucrose, starch and cellulose based products. For cellulose based products, because of the complex and crystalline structure of lignocellulose, it is more difficult to hydrolyze than starch. But with the use of some thermostable cellulase, hemicellulase and thermophilic organisms, the degradation of lignocellulosic material to ethanol can be obtained by a single step enzymatic process and also minimizes the risk of contamination. Maximum cellulases production has been found from *Anoxybacillus flavithermus*, *Geobacillus thermodenitrificans* and *Geobacillus stearothermophilus*[58].

Other Applications

a. Degradation of Prion Proteins: Prions are infective agents of fatal neurodegenerative diseases called transmissible spongiform encephalopathies (TSE). This includes Bovine Spongiform Encephalopathy in cattle, Scrapie in sheep and goat and Creutzfeldt-Jakob disease in human. Infection of prion is accompanied by conversion of harmless PrPc to infectious PrPsc protein. A thermophilic keratinase from *Bacillus* sp. WF146 disintegrates prion protein at 80°C[39]. These enzymatic methods have also been used in the decontamination of precision instruments that are susceptible to prion contamination[74].

b. In Bioremediation: Bioremediation can be defined as a pollution treatment technology that exploits microorganisms to reduce, eliminate, contain and transform environmental contaminants to benign products[64]. Microorganisms inhabiting contaminated environments, therefore, have evolved various survival strategies to restrict intracellular metals within permissible limits. Owing to their small size, high surface to volume ratio and metabolic versatility, microbes can frequently interact with these contaminants that strongly regulate environmental fate of metals by altering their physical and chemical states and therefore their solubility, mobility, bioavailability and toxicity[6, 25].

Table 6
Important Thermozymes and their Industrial Applications[18]

Enzyme	Thermophilic Bacteria	Temp °C	Bioconversions	Industrial application
Amylase	*Bacillus sp.*	80-100	Starch to dextrose	Biofuel, Baking, Brewing and Health care
Pullulanase	*Pyrococcus furiosus*	50-60	Starch to dextrose	Food and Beverage
Cellulase	*Geobacillus sp.*	55-65	Cellulose to dextrose	Biofuel, Detergent, Textile
Xylanase	*Thermotoga sp.*	45-65	Hemicellulose to xylose	Paper and pulp
Protease	*Thermococcus* and *Pyrococcus sp.*	65-85	Peptide to amino acids	Detergent, Pharmaceuticals, Food and beverage, Leather
Lipase	*Geobacillus sp.*	40-70	Trans esterification, fat hydrolysis	Dairy, Cosmetic, leather, Pharmaceutical
DNA Polymerase	*Thermus aquaticus*	90- 100	DNA amplification	Biotechnology, PCR

Table 7
Recent Patents on Thermophiles and their Potential Applications

S.N.	Topic	Patent number and date	Application
1	Single step bioconversion of lignocellulosic biomass to biofuels[15]	US2014/0363869 A1 December 11, 2014	Bioconversion of lignocellulosic biomass to biofuels
2	Thermophilic bacterium and uses of extracellular proteins[30]	US 8828238 B2 September 9, 2014	Excellent metal ion binding ability
3	Fermentation of moderately thermophilic Bacilli on sucrose[33]	US 8,663,954 B2 March 4, 2014	Genetic modification of moderately thermophilic Bacillus strain to utilise sucrose as a carbon source
4	Bioremediation of persistent organic pollutants[48]	US 2014/0042087 A1 February13, 2014	Degradation of organic pollutants
5	Phytase-producing bacteria, phytase and production method of phytase[13]	US 6,180,390 B1 January 30, 2001	Role in animal feeding, environmental protection, human nutrition , health and industries
6	Process for producing modified microbes for oil treatment at high temperatures, pressures and salinity[19]	US 5492828A February 20, 1996	Used in microbial enhanced oil recovery

Thus, thermophilic microbial metal resistance and interactions offer enormous opportunities to develop bioremediation strategies for cleaning up of contaminated environments associated with high temperature and other stressful factors**.** They are thus used during bioremediation of hot wastewater of disposal sites of radioactive wastes having temperature range favorable for thermophiles for a long period of time[59].

c. Astrobiology: Thermophiles are organisms that survive at high temperatures and studying these organisms especially their protein stability is important as they can tell us what extraterrestrial life will look like. Astrobiologists study the protein structural motifs of the thermophiles to hypothesize and investigate the possibility of extraterrestrial life forms and early life. By studying the motifs, they are able to say if organisms similar to the thermophiles are able to survive in planets that have a hot environment like those found in the deep-sea thermal vents. It is assumed that the mechanism adopted by thermophilic organism to survive the extreme conditions opens new windows to how organisms are able to survive in very extreme environments[44].

Limitations of Thermophilic Bacteria

Even though the use of thermophiles at high temperatures is economically attractive, the biomass achieved by these organisms is inadequately low compared to mesophiles. Special equipments, media composition and specific processes are being developed to improve the biomass yield of thermophilic organisms. But their large scale cultivation still remains an economical challenge due to factors like low growth rate, requirement of complex, expensive media and low solubility of gases at high temperature. The high cost of production of thermophiles is justifiable for very few applications. To reduce the production cost, many thermostable enzymes have been cloned and successfully expressed in mesophilic organisms. Tools for the over expression of these enzymes in thermophilic and mesophilic hosts are being developed to meet the increasing demand for biocatalysts. These enzymes could also serve as models to understand molecular stability under extreme conditions.

Conclusion

Thermophiles have been a topic of much interest because of their ability to survive in harsh temperatures. They are much studied to utilize their adaptive mechanisms for commercial purposes. Thermophilic organisms produce various valuable compounds. These enzymes have wide ranging applications like in crop biorefining, food and pharmaceutical, detergents, textile and several other biotechnological industries. Other applications of these organisms include potential for electricity generation, herbicide metabolism and production of exopolysaccharides of commercial importance. It is believed that the study of these organisms will enhance the existing knowledge on astrobiology as well as origin of life on earth.

The increasing number of patents indicates that there is a growing interest in the commercial applications of thermophiles. The demand for thermostable enzymes has increased tremendously in the past few years. Since only a very few species from this group of microorganisms have been isolated till date, there seems to be a large number of hyperthermophilic catalysts with unique properties awaiting discovery.

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