

THERMOPHILIC BACTERIA AND THEIR POTENTIAL FOR INDUSTRIAL APPLICATIONS

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Abstract: Enzymes produced by thermophilic bacteria have great potential in industrial biotechnology due to their ability to withstand wide range of temperature extremes. They have also gained industrial importance because of the improvement and invention of various tools and technologies. Thermozyms serve as industrial enzymes superior to traditional catalysts, as they perform better in high temperature conditions required for various industrialized enzymatic processes. As a result, the characterizations of microorganisms that are able to thrive in high temperature conditions have received a great deal of attention of researchers and industrialist all over the world. This review discuss about the different uses of thermophilic bacteria in various industrial applications such as in enzyme industry, in medicines, in single cell protein production, in bioconversion of wastes, in petroleum industry, in fuel production, in mining, and as biosensors. Also, this review focuses on phylogeny and stabilizing factors responsible for the existence of these bacteria under thermal environmental conditions. Although, a lot of work has been done to understand biochemical basis of thermophily, still further research work is required with respect to the genomic and proteomic features of thermophilic bacteria and their enzymes of industrial interest.

Keywords: High-temperature catalysis, Industrial applications, Thermophilic bacteria, Thermophily

INTRODUCTION

Thermophilic bacteria are those organism which thrive at relatively high temperatures, between 45 and 80°C (113 and 176°F) and are an important group of organisms, which have tremendous potential in various biotechnological and industrial applications. Thermophilic bacteria were first isolated in 1879 by Miquel (Miquel, 1888), who found bacteria capable of developing at 72°C (162°F); and from that day till today, a variety of thermophilic bacteria have been isolated from various geothermally heated regions of the earth such as hot springs, deep sea hydrothermal vents, hot desert soil and compost piles etc. Enzymes of these thermophilic bacteria exhibit inherent thermostable property thus well suited for high-temperature catalysis of various industrial processes. The benefit of using thermozyms in industrial processes are manifold, including reducing risk of contamination, higher reaction rates, higher solubility of most chemicals and increased fluidity and diffusion rates. Thermophilic bacteria have been less explored and investigated due to hurdles in isolaion and maintenance in pure culture and further greater challenge for their survival and production of active and stable enzymes. Still a number of research

publications have extensively discussed development in area of thermozyms from thermophilic bacteria, adaptation to high temperature (Danson *et al.*, 1992; Stetter, 1999), production of heat stable enzymes from thermophiles and biotechnological and industrial applications of thermophilic bacteria (Cowan, 1996; Host *et al.*, 1997).

PHYLOGENY OF THERMOPHILIC BACTERIA

The five Kingdom classification systems of organisms: The Monera (bacteria and bacterium-like organisms), representing prokaryotes and protists (mainly unicellular nucleate organisms), fungi, plants and animals, representing eukaryotes given by R.H.Whittaker in 1969 have tendedly recognized by biologist in recent years. But being very simple and not phylogenetic, the five kingdom classification has significant demerits. Earlier natural classification, based on evolutionary relationships, was not possible with bacteria but in last few years with the possibility of sequencing nucleic acid the scenario has been changing rapidly. Nucleic acid sequencing has provided a new approach for studying evolutionary relationships, since all organisms have a genome, the genes that code for vital cellular functions remains

very much conserved to a remarkable degree. Thus, by comparing such genes, it should be possible to assess the relatedness of different organisms. The gene most commonly used for this purpose are the genes code for 16S rRNA present in small subunit (SSU) of the ribosome. It is clear from such analysis that thermophilic bacteria are distributed among all the main groups in bacterial tree. In the current edition of Bergey's manual the prokaryotes, the bacteria culturable in laboratory are distributed among 26 phyla. Out of these 26 phyla, 24 belong to domain bacteria and 2 phyla to domain archaea. Among 24 phyla of domain bacteria, six phyla including Aquificae, Thermotogae, Thermodesulfobacteria, Dictyoglomi and Thermomicrobia contain exclusively thermophiles and 11 phyla including Chloroflexi, Deinococcus-Thermus, Nitrospirae, Deferribacters, Proteobacteria, Firmicutes, Actinobacteria and Spirochaetes (Lebedinsky *et al.*, 2007) contain thermophiles along with mesophiles. What is particularly interesting, however, is that thermophiles are usually the oldest types in their respective groups and even the oldest of the bacterial kingdom (Miquel, 1888). This suggests that thermophily as special property developed very early in the evolution of life on earth.

STABILIZING FACTORS

Some of the possible mechanism leading to thermophilic existence of a variety of system including enzymes, as proposed by various workers involve lipid and membranes, highly charged macromolecular environment, allostery, thermo-adaptation, transformation, intraprotein interactions, polar surface area, stability of secondary structural elements, cavity size and amino acid composition and other genomic and proteomic features (Singh, 1980; Hawwa *et al.*, 2009). Also, a slight difference among homologous enzymes can account for differential thermostability within a superfamily (Goihberg *et al.*, 2007). On the basis of comparative study of lipids of thermophilic and mesophilic microorganisms, Helibrum (1924) and Belehradek (1931) have suggested that higher melting points of lipids in the former may be responsible for their thermophily. It has been reported that the α -amylase from a facultatively thermophilic *Bacillus* species grown at 55°C is thermostable and the enzyme is thermolabile when the bacterium is grown at 37°C (Campbell, 1955). Williams (1975) has pointed out that the mechanism of thermophily can be easily ascertained if the facultative thermophils are

employed for a direct comparison of the enzymes from thermophilic and mesophilic variants of the same organism. Genetic transformation has also been used for a better understanding of the cause of survival of thermophiles at high temperatures (Amelunxen and Murdock, 1978). The mesophilic *B. subtilis* becomes thermophilic as a result of transformation by *B. caldolyticus* donor DNA (Lindsay and Creaser, 1975). In thermophiles the genomic and proteomic features are marked by overrepresentation of purine bases in protein coding sequences (Lao and Forsdyke, 2000), over presentation of GC bases in the structural RNAs, presence of higher tRNA diversity (Satapathy *et al.*, 2010), presence of distinct synonymous codon usage, enhanced usage of positively charged residues and aromatic residues and decrease in polar uncharged residues in the encoded protein (Das *et al.*, 2010) and presence of chaperones in thermophiles (Demirjian *et al.*, 2001). DNA supercoiling in thermophiles plays essential role in maintaining equilibrium between genome dynamics and stability (Valentil *et al.*, 2011).

ROLE IN THERMOZYMES PRODUCTION

Enzymes occur ubiquitously in animals, plants and microbes. However microbes represent the preferred source of industrially important enzymes in view of their rapid growth, limited space required for cultivation and easy accessibility for genetic manipulation. Various thermostable enzymes like amylases, proteases, lipases, xylanases, and cellulases have their biotechnological potential for applications in detergent, food, feed, textile, leather, paper and pulp and pharmaceutical industry.

Microbial proteases account for approximately 40% of the total world wide enzyme sale (Godfrey and West, 1996). The majority of proteolytic enzymes are produced by the member of the genus *Bacillus* (Samal *et al.*, 1989). Subtilases are a family of subtilisin-like serine proteases utilized extensively in detergent industry (Poul, 2010). A novel subtilase has been characterized in the spores of *Thermoactinomyces* sp. CDF (Cheng *et al.*, 2009). Commercial proteases like thermolysin and SP 369 are produced by *Bacillus thermoproteolyticus* and *B. stearothermophilus*, respectively (Endo, 1962; Zamost *et al.*, 1990). Amylases alone form approximately 25% of the enzyme market (Sindhu *et al.*, 1997). Some important sources of heat stable amylases are *Bacillus amyloliquefaciens*, *B.*

stearothermophilus, *B. subtilis*, *B. circulans*, *B. cereus*, *Clostridium thermosulfurogenes*, *Lactobacillus manihotivorans* and *Staphylothermus marinus* (Haki and Rakshit, 2003). In our laboratory optimization and characterization of thermophilic protease from *T. vulgaris* have been done (Singh, 2010). Lipases find immense biotechnological applications in detergent, oleochemical, dairy and pharmaceutical industry (Ching, 2000). Production of thermostable alkaline lipase on vegetable oils from *Bacillus* sp. DH4 shows its potential application as detergent additive (Bora and Kalita, 2008). Other sources of thermostable lipases are *Geobacillus stearothermophilus* strain-5 (Berekaa *et al.*, 2009), *Thermoanaerobacter thermohydrosulfuricus*, *Caldanaerobacter subterraneus* (Royter *et al.*, 2009) and *Bacillus* sp. isolate DM-15 (Bahri *et al.*, 2010). Xylanase and Cellulase, together with pectinase account for 20% of the world enzyme market (Polizeli *et al.*, 2005). Xylanases utilized extensively in bio-bleaching process of pulp and paper industry. Various thermophilic bacteria such as *Thermospora fusca*, *Thermospora chromogena*, *Bacillus* sp. and *Clostridium stercorarium* xylanase A are the important sources of thermophilic xylanases (Zamost *et al.*, 1991). Recently, a salt tolerant xylanase from *Thermoanaerobacterium saccharolyticum* NTOU 1 has characterised by Hung *et al.* (2011) which may be useful for various industrial applications. Thermostable chitinases suitable for bioconversion of chitin waste are produced by *Bacillus licheniformis* (Songsiriritthigul *et al.*, 2010), *Aeromonas* sp. DYU 007 (Lien *et al.*, 2007) and *Bacillus* sp. 13.26. (Yuli *et al.*, 2004). Taq polymerase, a thermostable DNA polymerase was first characterized from *Thermus aquaticus* in 1976 (Chien *et al.*, 1976; Kaledin *et al.*, 1980). Kary Mullis at Cetus corporation discovered that this enzyme could be used in the Polymerase Chain Reaction (PCR) process for amplifying short segment of DNA (Saiki *et al.*, 1988). Since a 3'-5' exonuclease activity was not detected in Taq polymerase thus the base insertion fidelity of this enzyme is low (Longley *et al.*, 1990; Ling *et al.*, 1991). It has been observed that PCR procedure can be optimized by mixing a standard polymerase, such as Taq polymerase with high fidelity polymerases (Ling *et al.*, 1991). Some important thermostable high fidelity DNA polymerases used in PCR are: Thermostable high fidelity DNA polymerase such as Bst I polymerase purified from *Bacillus stearothermophilus* (Mead *et al.*, 1991), Tfi polymerase purified from *Thermus filiformis* (Perler *et al.*, 1996), Tth polymerase purified from *Thermus thermophilus*

(Myers and Gelfand, 1991; Pantazaki *et al.*, 2002), and a new DNA polymerase patented from *Anaerocellum thermophilum* (Waltraud *et al.*, 2004), are being utilized in PCR process. Zotta *et al.* have successfully screened *Streptococcus thermophilus* for production of urease for removal of urea from alcoholic beverages (Zotta *et al.*, 2008).

ROLE IN MEDICINES

Thermophilic bacteria and their enzymes have tremendous role in medicinal field, including pharmaceuticals and antibiotic production. Production of antibiotic is a general phenomenon for most bacteria. The resistance among these microbial pathogens toward the antibiotics is increasing with great speed. As a result, productions of more rational approach to antibiotic use and new antimicrobial compounds have brought the attention of industrialist and scientist world widely. Among bacteria *Bacillus* species produce a vast number of antimicrobial compounds active against bacteria, fungi, protozoa and viruses. It has been reported that *Bacillus* sp. SAT4 remains active in fermentation process to produce the microbial agents and this species was found to be active against *Micrococcus luteus*, *staphylococcus aureus* and *Pseudomonas aeruginosa* (Ahmad and Hameed, 2009). Many antibiotics are produced from different strains of thermophilic actinomycetes, for example Thermomycin from *Streptomyces thermophilus*, Thermorubin from *Thermoactinomyces antibioticus*, T-SA-125 from *Thermospora* sp., Thermovirdin from *Thermoactinomyces viridis*, Thermothiocin from *Thermoactinopolyspora cormialis* and Granaticin from *streptomyces thermoviolaceus* (Edwards, 1993). Baciamin, an antifungal protein was isolated from *Bacillus amyloliquefaciens*. Baciamin also exhibited other exploitable properties such as antitumour and immuno-enhancing activities (Wong *et al.*, 2008). Several genera of thermophilic bacteria and their enzymes have been in use in various pharmaceutical applications all over the world. A purified strong fibrin specific fibrinolytic enzyme is obtained from thermophilic *Streptomyces megasporus* SD5 (Chitte and Dey, 2000). A novel enzyme actokinase was extracted from this bacterium and used for treatment of thrombolytic disorders (Chitte and Dey, 2001). Another enzyme inulinases used for production of fructose and fructooligosaccharides, which are extensively used in pharmaceutical and food industry. The thermostable inulinases have reported from thermophilic bacteria as well (Singh and Gill, 2006). The

thermophilic treatment of bulk drug pharmaceutical industrial waste water is also reported by Sreekanth *et al.* (2009).

ROLE IN SCP (SINGLE CELL PROTEIN) PRODUCTION

In general usage SCP is a microbial protein or microbial food used as a substitute for protein rich foods in human and animal feeds. Among the different sources of protein including fish protein, leaf protein, milk protein, oil seed protein etc., the SCP is one of them. SCP has been produced by aerobic fermentation processes at relatively high fermentation temperature conditions employing a unique thermophilic mixed culture of bacteria NRRL B-8158 as microbial conversion agent (Hitzman and Okla, 1981). It has been found that *Thermoactinomyces sp.*, *Myrothecium verrucaria* and *Saccharomyces cerevisiae* can be used to produce SCP (Humphery *et al.*, 1975; Vyas and Deshpande, 1991). Various microbial enzymatic processes such as bioconversion of cellulose to glucose open up new avenues of their industrial applications, including SCP production (Ishaque and Kluepfel, 1980).

ROLE IN BIOCONVERSION OF WASTE

The bioconversion of waste is one of the important components of the sustainable development in which waste can be converted into useful product with the help of living organisms such as thermophilic bacteria. It has been reported that the bioconversion of food waste and sewage sludge becomes most effective with thermophiles, when pH is buffered with calcium carbonate (Yuan *et al.*, 2003). The investigation of microbial communities present in three fuel scale anaerobic thermophilic digesters, which treated sewage sludge, garbage and livestock wastes using 16S rDNA clone libraries resulted that *Bacillus infernus* and *Methano thermobacter wolfeii* recommended as key microbes in thermophilic garbage digester (Cheon *et al.*, 2007). In hot compost at temperature between 65-80°C vast numbers of aerobic thermophilic bacteria are found. The presence of functional bacterial diversity during the thermogenic phase makes compost possible at high temperature (Beffa *et al.*, 1998). Various spp. of Thermoactinomycetes such as *Thermomonospora curvata*, *Thermomonospora fusca*, *Thermomonospora chromogena*, *Thermoactinomyces vulgaris* and *Thermoactinomyces thalophilus*, are found in compost for mushroom; *Thermoactinomyces vulgaris*, *Thermomonospora alba*, *Thermomonospora cur-*

vata and *Thermomonospora lineata* are found to be grown in domestic waste compost; *Thermoactinomyces vulgaris* is also found in green waste compost and sewage compost (John lacy, 1997).

ROLE IN PETROLEUM INDUSTRY

Microorganisms are also involved in such diverse field of industries as petroleum formation; clean up of oil spills, exploration of petroleum and deterioration of petroleum products. It is reported that microbes including thermophilic bacteria can utilize hydrocarbons as sole source of carbon and energy and can degrade petroleum hydrocarbons both in marine and fresh water ecosystems. Oil spill is one of the major causes of water pollution in oceans. Researches have been conducted all over the world to find out the biological method utilizing bacteria for oil spill degradation. In this quest in U.A.E. researchers have isolated several strains of thermophilic bacteria having potential of bioremediation of oil spills both in in-situ and ex-situ conditions and in microbial enhanced oil recovery. These bacteria found to be active at 80°C for breaking down long chain organic molecules (AlMaghrabi *et al.*, 1998). Another research group in Kuwait observed that desert samples contaminated with crude oil containing *Bacillus stearothermophilus* were capable of growing at high temperature on crude oil as a sole source of carbon and energy (Sorkhoh *et al.*, 1993). The hydrocarbon degrading species of *Bacillus* and *Geobacillus* could be effectively used for bioremediation of oil polluted soil (Meintanis *et al.*, 2006).

ROLE IN FUEL PRODUCTION

Due to oil crisis there is increased interest in use of alcohol, hydrogen and biogas as a supplement to conventional fuels. The biogas, hydrogen and ethanol are important fuel products obtained through fermentation process utilizing thermophilic microorganism such as thermophilic bacteria. The usage of fuel Gasohol (mixture of 90% unleaded gasoline and 10% alcohol) is dependent on the cost of producing alcohol. Since large quantities of suitable raw material are available for fermentation, this fuel can be easily produced, using thermophilic bacteria, for example, *Thermoanaerobacter ethanolicus* is commonly used in ethanolic fermentation (He *et al.*, 2009). It has been reported that the group of extreme thermophilic bacterial species related to *Thermoa-*

naerobacter, *Thermoanaerobacterium* and *Caldanaerobacter* involved in simultaneous hydrogen/ethanol production (Zhao *et al.*, 2009). Production of ethanol by thermophilic fermentation of xylose and glucose is performed by *Thermoanaerobacterium ihermosaccharolyticum* w16 (Ren *et al.*, 2008). In another report, production of hydrogen from polysaccharide such as cellulose with the help of thermophilic bacterium *Clostridium thermocellum* has reported (Magnusson *et al.*, 2009). *Clostridium thermocellum* converts cellulose to hexose, ethanol, lactate, acetate and carbon dioxide (Weimer, 1986).

ROLE IN MINNING

The traditional method of processing ores is a major cause of air pollution. In recent years, microorganisms have been used in the process of recovery of minerals from ores without any pollution; thus, such process is known as biohydrometallurgy. It concerns with geologists, economic geologists, metallurgists, chemical and mining engineers, hydrometallurgist, chemists, and most importantly the microbiologists. Biohydro- metallurgy is mainly used to recover certain metals from sulfide ores. It is very much useful in recovering metals such as copper, gold, lead, nickel and zinc (Rossi, 1990). Nowadays microorganisms are used in copper mining and refractory gold pretreatment. The commercial viability of utilizing moderate thermophilic bacteria to recover the solubilised copper with solvent extraction electrowining has been demonstrated at copper mines of Australia's Mt. Lyell operation in Tasmania (Rhodes *et al.*, 1998). Refractory gold pretreatment, using biooxidation heap technology can be performed at 60°C to 75°C (Brierle, 1997), which necessitates the use of thermophiles that can function from 45°C to over 70°C. The report published reflects the growing interest in basic and practical application studies of bioleaching using thermophilic microorganisms (Rivera *et al.*, 1999; Rawlings, 2002).

ROLE AS BIOSENSORS

Biosensors consist of a biological entity that can be an enzyme, antibody, or nucleic acid that interacts with an analyte and produces a signal that is measured electronically. Each biosensor, therefore, has a biological component that acts as the sensor and an electronic component to transduce and detect the signal. A variety of substances including nucleic acids, proteins (particularly antibodies and enzymes), lectins (plant proteins that bind sugar moieties) and

complex materials (organelles, tissue slices, microorganisms), can be used as the biological components. In each case, it is the specificity of the biological components for an analyte (or group of related analytes) that makes the biomolecules attractive as sensing component (Verma and Singh, 2005). A microbial sensor for BOD (Biochemical Oxygen Demand) measurement was developed by utilizing thermophilic bacteria isolated from Japanese hot spring (Karube *et al.*, 1989). Koblizek *et al.* (1998) have developed a biosensor for the detection certain herbicides, using isolated photosystem II (PSII) particles from the thermophilic cyanobacterium, *Synechococcus elongatus*, as biosensing elements.

CONCLUSION

Since mid 1980s, there has been genuine growth in industrial use of thermophilic bacteria. Commercialization/industrial uses reflect the realization by proper management that thermophilic bacteria are robust and economically very useful. They work at relatively higher temperature so they led to higher reaction rates, higher solubility of most chemicals and increased fluidity and diffusion rates etc. Thus, the toolbox to select and make use of these organisms is steadily expanding. With the exploration of new biotechnological techniques, these organisms show tremendous potential in economic climate of various industrial uses such as in antibiotic, fuel and single cell protein production, in bioconversion of waste, in oil spill degradation, in pharmaceuticals, in deterioration of materials, in biohydrometallurgy and as biosensors.

Table 1. Some of the industrially important enzymes and antibiotics with their microbial sources.

Some industrially important enzymes from thermophilic bacteria		
Source organism	Enzyme	Reference
<i>Bacillus thermoproteolyticus</i>	Thermolysin	Endo, 1962
<i>B. stearothermophilus</i>	SP 369	Zamost <i>et al.</i> , 1990
<i>Bacillus</i> sp. DH4	Lipase	Bora and Kalita, 2008
<i>Thermoanaerobacterium saccharolyicum</i> NTOU 1	Xylanase	Hung <i>et al.</i> , 2011
<i>Bacillus licheniformis</i>	Chitinase	Songsiriritthigul <i>et al.</i> , 2010
<i>Thermus aquaticus</i>	Taq polymerase	Chien <i>et al.</i> , 1976; Kaledin <i>et al.</i> , 1980
<i>Thermus thermophilus</i>	Tth polymerase	Myers and Gelfand, 1991; Pantazaki <i>et al.</i> , 2002
<i>Bacillus stearothermophilus</i>	Bst I polymerase	Mead <i>et al.</i> , 1991
<i>Thermus filiformis</i>	Tfi Polymerase	Perler <i>et al.</i> , 1996
<i>Streptococcus thermophilus</i>	Urease	Zotta <i>et al.</i> , 2008
Some important antibiotics from thermophilic bacteria		
Source organism	Antibiotic	Reference
<i>Streptomyces thermophilus</i>	Thermomycin	Edwards, 1993
<i>Thermoactinomyces antibioticus</i>	Thermorubin	
<i>Thermoactinomyces viridis</i>	Thermovirdin	
<i>Thermoactinopolyspora cormialis</i>	Thermothiocin	
<i>Streptomyces thermoviolaceus</i>	Granaticin	

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