Available online at www.ewijst.org

ISSN: 0975-7112 (Print)
ISSN: 0975-7120 (Online)

Environ. We Int. J. Sci. Tech. 5 (2010) 27-34

Environment & We
An International
Journal of Science
& Technology

Hyperthermophiles: the Pioneeres of This Planet and for Global Research Pioneers

Prakash Chandra Tiwari[1], Chandra Bhan Chaudhary[1],
S. Shukla[1] and Sanjeev Kumar Srivastava[2]*
[1]Department of Environmental Sciences, Dr. R. M. L. Avadh University,
Faizabad-224001 (U.P.) India
[2]Sri Ram Murti Smarak College of Engineering & Technology,
Bareilly-243202(U.P.) India.
*E-mail: sanjsri2001@yahoo.co.in

Abstract

These organisms live in the biotype with several stress factors like extreme high and low temperature, pressure, salt concentration etc., and come under the archaebacteria of kingdom Monera. An enhancement in their properties gives us new dimensions which were widely been used for global environmental cleanup, pollution prevention, energy productions and in creating the model of probabilities, extraction of important macromolecules and chemical needed for survival of micro organisms. These organisms provide an inspiration for understanding fundamental questions of environmental sciences, biochemistry, biotechnology and nanotechnology. The current need is to emphasize more global research programs to understand their importance in evolution, possibilities of developing cost effective eco-friendly remediation processes and economical utility for humanity.

Key Words: Extremophiles; Chemo-organotropic; Thermostability; Proteolytic enzymes; Amylolytic.

Introduction

Those microorganisms, which thrive under intolerable hostile environment where other microbes never ever dare to venture, are known as extremophiles. The term extremophiles was firstly introduced by R. D. MacEloroy in 1974 (Hendry, 2006). Their habitat ranges from volcanic vent of Hawaii, deep abyssal of oceans like pacific, frozen ice capes of Antarctica, flow of Gangotri glacier of Western Himalayan (Baghel *et al.,* 2003), geysers of Yellow Stone National Parks which are high in hydrogen sulphide, iron, hydrogen and carbon dioxides and even on the Moon, the probability may indicate their presences on Europa and Mars. These organisms live in the biotype with several stress factors like extreme high and low temperature, pressure, salt concentration etc., and come under the archaebacteria of kingdom Monera. They were classified, according to the environmental conditions in which they exists such as, thermophiles (*Geobacillus stearothermophilus, Geobacillus thermooleovorans, Dictyoglamous thermophilum*), hyperthermophiles (*Archaeoglobus profundus, Pyrolobus fumarii, Ignicoccus islandicum*), psychrophiles *(Lifsonia aurea, Sporosarcina macmurdoensis, Kocuria polaris*), acidophiles (*Dunaliella acidophila, Alicyclobacillus acidocaldarius, Thiobacillus ferrooxidans*), alkaliphiles (*Exiguobacterium aurantiacum, Ancylobacterium* sp., *Flavobacterium* sp.), barophiles (*Photobacterium, Shewanella, Alteromonas* sp.) and halophiles (*Fabrea salina, Debaryomyces hansenii, Ulocladium clamydosporum*) (Satyanarayana *et al.,* 2005).

The most interesting group of the extremophile is hyperthermophiles. Those organisms which thrive under extremely high temperature more than 80^0C are known as hyperthermophiles. They come under kingdom Crenarchaeota. These hyperthermophiles were first discovered in hot spring in Yellowstone national park in 1966 by Thomas Brock (Maloney, 2003). Now a dayõs researches were emphasized over these hyperthermophiles having ability to tolerate high temperature with unique genetic and biotechnological features.

Till date known habitat of these organisms on this planet are continental and submarine volcanic areas, such as solfatar fields (steam heated soils, mud holes), surface waters of deep hot springs, geothermal power plants as well as submarine hot springs and fumaroles. By studying their biotechnological aspect, the new genetic strains were obtained which are more dominant as compare to other micro organisms of this planet.

An enhancement in their properties gives us new dimensions which were widely been used for global environmental cleanup, pollution prevention, energy productions and in creating the model of probabilities, extraction of important macromolecules and chemical needed for survival of micro organisms. These organisms provide an inspiration for understanding fundamental questions of environmental sciences, biochemistry, biotechnology and nanotechnology.

Physiological and Morphological Characteristics:

Most of the anaerobic hyperthermophilic bacteria are chemo-organotropic in their metabolism. These bacteria belong to the same range of nutritional categories as mesophilic bacteria. The physiological process for adaptation to environmental stress in anaerobic bacteria seems to have evolved in different factors from those in aerobic bacteria. These hyperthermophiles archae have almost the same size as one typical prokaryotic cell. *i.e.*, 0.5 µm in length and 2.0 µm in width, some of them have their own unusual morphological feature. They include methanogens, sulphate reducers, nitrate reducers and also aerobic respirators. However, majority of species known at present is strictly anaerobic heterotrophic reducers.

All of the hyperthermophilic heterotrophs can use complex peptide mixture, like peptone, tryptone or yeast extract as carbon and energy sources.

Hyperthermophiles are rod shaped enclosed by a sheath like envelop called toga. The cell envelop of these bacteria consists of the cytoplasmic membrane of lipid containing long chain of dicarbocylic space and surface protein, with ultra thin sections are also reveled with a zigzag structure of S-layer. These S-layer proteins are highly stable and maintain the structural integrity of bacterial cells under extreme environmental conditions and resists dissociation by high temperature, chemical treatment or mechanical disruptions. These hyperthermophiles also have ability to grow at low and high salt concentration. The most interesting fact regarding their niche is that, there are no any competitions for needs within the colonies or species (Andrade *et al.,* 1999).

A functional genomic approach is being used to study three model hyperthermophilic microorganisms *Pyrococcus furrosus, Thermatoga martima* and *Methanococcus jannaschi,* that have complementary physiological characteristics and for which complete genome sequencing data are available. These hyperthermophiles are being examined individually and the mixed cultures for physiological characteristic that are related to physical, chemical and biological aspects of their growth in environment, inter-intra specific interaction and propensity for forming biofilm. In the same order one of the most heat tolerant hyperthermophiles is recently discovered Strain 121 which has been able to double its populations during 24 hours in an autoclave at 121^0C (Ferrer *et al.,* 2004).

Biotechnological aspects

The biotechnological potential of hyperthermophiles is increasing day by day with isolation and characterization of new organisms with their cellular components. The first archae bacteria *Thermococcus littoralis* related products are enzymes in the form of DNA polymerases which is now being used for global research and are helpful for decoding or sequencing large block of all kind of DNA. The current biotechnological interest from these micro organisms is motivated by their ability to work under conditions that are normally denaturing other mesophilic enzymes.

The hyperthermophiles have capability to synthesize heat stable molecules such as enzymes and have both intracellular and extracellular enzymatic activity which show not only great thermostability but also enhanced their activity in presence of common proteins denaturants such as detergents, organic solvents, and proteolytic enzymes (Andrade *et al.,*1999). The 16 S r-RNA genome sequences with 1,416 bases of strains B1001was isolated from hot spring environment of Japan had a Guanine + Cytosine content of 43.0 mole% as calculated by direct analysis of nucleotide.

It is capable of growing on proteinaceous complex of substrates such as yeast extract, peptone and tryptone. They are irregular cocci of 0.5µm to 1.0µm in diameter obtain from hyperthermophile genus Thermococcus produce cyclomaltodextrin glucanotransferase (CGTase) and was purified 1,750 folds with molecular mass of 83 kDa. The optimum temperature for starch degrading activity and cyclodextrin synthesis activated at 100^0C to 110^0C. The CGTase enzymes are able to form inclusion complexes with many organic and inorganic molecules, thereby changing physical and chemical properties of included compound.

On behalf of biotechnological ground enzyme CGTase are used for catalysis of four different transferase reactions like cyclization, coupling, disproportionation and hydrolysis (Tachibana *et al.,* 1999). Therefore CGTase is now an important enzyme used in food, cosmetic and pharmaceutical industries.

The two clones of xylananses obtained from hyperthermophile, *Dictyoglomus thermophilum*, *Kluyveromyces lactis* and *Trichoderma reesei* were obtained to immobilize thermostable molecular chaperonins CpkA and CpkB of hyperthermophilic archaeon thermococcus *Kodakaraensis* (KODI) that are reported to be useful in enzyme stabilization (Satyanarayana *et al.,* 2005). An enzyme alcohol dehydrogenase (ADH) derived from hyperthermophile *Sullfolobus solfataricus* that works under some of natureȸs harshest volcanic conditions, these enzyme catalyses the conversion of alcohol several bio-molecules obtained from these hyperthermophiles are stable at high temperatures and are capable to degrade normal cellular molecules such as enzymes, lipids and nucleic acids.

Establishment of genome sequence database and genome based technologies, coupled with the construction of recombinant micro organisms with surely derive typical examples of shift in programs regarding discoveries of antibiotic This characters lies in their extremoenzymes, (enzymes which geared to work in extremely high temperature) and have special characters to retain their twisted and folded 3D structures of amino acid. More than 50 species of hyperthermophiles have been isolated, for endoglucanases, exoglucanases, cellobiohydrolases, xylanases, ß-glucosidase and ß- galaclosidase, which are resistance to boiling temperatures (Hongpattaraker, 2002).

Recently, *Nanoarchaeum equitans* also known as ancient dwarf who rides the fire ball was been discovered by Karl Stetter and his team of microbiologist this is the worldȸs smallest microorganism. This hyperthermophiles was found in 120m depth submarine hydrothermal vent, of north Iceland and thrives in temperature close to 100^0C. This discovery created a mile stone in the field of nanotechnology and led to the creation of new phylum Nanoarchaeota. With less than 500 kb in its genome, *Nanoarchaeum equitans* represents the smallest genetic code of all living organisms to date.

The importance of such novel microorganism is highlighted by the Californian Biotech Company - Diversa Corporation. This company gained exclusive right to all commercial applications derived from this new hyperthermophiles (Maloney, 2003). These capability of hyperthermophiles open new avenue for their biotechnological enhancement in characteristics and used for desired applications like production of new pharmaceutical, antibiotic, preservation of food materials, cosmetics and best used as pollutant scavengers.

DNA stability at extreme temperature

Several investigations regarding maintenance of DNA structure at extremely high temperature shows that the primary DNA structure faces more difficulties as compared to secondary one, though hyperthermophiles have modest level of spontaneous mutation within themselves. Their radiation sensitivity indicated that hyperthermophiles have capabilities to repair their DNA efficiency in vivo (Grogan,

1998) such as *Deinococcus radiodurans* which is multigenomic resistance to mutagenic chemicals, UV, IR-radiations and desiccation (Satyanarayana *et al.,* 2005).

There are several enzymes showing amylolytic activity for DNA metabolism, which have unusual biochemical features that may ultimately prove relevant for DNA repair in hyperthermophiles (Tachibana *et al.,* 1999). The hyperthermophiles are now well characterized taxonomically at the level of DNA-DNA hybridization and their proposed evolutionary relatedness. The unique DNA technology in hyperthermophiles appears with the need of organisms to maintain integrity for their genome at high temperature.

A number of DNA binding proteins has been isolated from other hyperthermophiles but not yet been tested so much to detects their effect on DNA topology. A DNA binding histone protein HMf (5- hydroxymethyl 1 -2- furaldehyde) protein was obtained from hyperthermophile *Methanothermus fevidus* of ~7.5 Kda, which is capable of contrasting positive DNA super coils and was used to determine resemblance in nucleio-protein complex with super coiled plasmid, which was not denatured when tested at high temperature (Mai *et al,*, 1998). However, it was progressively transferred into open DNA by cleavage and then denatured due to its thermo degradation ability which is not dependent on the DNA super coiling density (Satyanarayana *et al.,* 2005).

Major DNA binding proteins, designated Ssh-7 which is capable of contrasting negative super coils, a property that has not been shown from any other DNA binding proteins was purified from the thermoacidophilic archaeon *Sulfolobus shibatae*. The Ssh-7proteins have an apparent molecular mass of 6.5kDa and are similar to the 7-kDa DNA binding proteins from *Sulfolobus acidocaldarius* and *Sulfolobus solifataricus*. These proteins are extremely stable to heat, acid and chemical agents. Sac7d binds to DNA without any particular sequence preference and thereby increases its melting temperature by approximately 40^0 C. Now, it is solved and refined that crystal structure of Sac7d is a complex with two DNA sequences at high resolution. These structures are examples of a nonspecific DNA-binding protein bound to DNA, and reveal that Sac7d binds in the minor groove, causing a sharp kinking of the DNA helix that is more marked than that induced by any sequence-specific DNA-binding proteins.

The results obtained from the intercalation of specific hydrophobic side chains of Sac7d into the DNA structure, but without causing any significant distortion of the protein structure it relative to the uncomplexed protein in solution (Robinson *et al.,* (1998). Though general mechanism for thermo protection of DNA in active cells is still unknown in hyperthermophiles, but the reverse gyrase is the only protein was reported which have both specific and common to all hyperthermophiles and reduces the rate of double stranded breakages upto 8-folds at 90^0C without ATP enzymes (Mai *et al.*, 1998).

The reverse gyrase have minor non specific effect at the rate of depurination but they have major specific effect on the rate of double strand breakage. This reverse gyrase stabilize the DNA backbone at site of damage and repair by cellular machinery (Atomi, 2005). Although the primary physiological role of reverse gyrase enzymes may be the control of DNA topology, reverse gyrase could take over protective functions whenever necessary. It was observed first time that topoisomerase and DNA accompanied together actively and combined in single enzymes that are reverse gyrase (Kampmann, and Stock, 2004). The major reason for targeting wide range of enzymes is their suitability as model for investigating proteins, thermostability with their potential as biocatalyst in modern biotechnology. Ground breaking research on hyperthermophiles continues till this day, with the recent discovered 22 genetically encoded aminoacids such as pyrolysine from *Methanosarcina barkeri* (Hendry, 2006).

Applications

There were many applications of hyperthermophiles present now a dayǿs or yet to be found, few important were listed as under below:

1. These microorganisms were helpful in understanding the evolution on our planet as per NASA with several other national as well as international research organizations reported that the first sign of life during evolution was started in high temperature.
2. An extracellular hydrolytic enzymes pullunase has been found in the culture medium after fermentation of starch by *Thermococcus hydrothermalis* which is used in combination with saccharifying amylase for the improved production of various sugar syrups (Andrade *et al.,* 1999).
3. They have been reorganized as valuable source of novel byproducts like strain Thermococcus used by PHENOMICS $_{R.}$ They have wide range of antimicrobial properties that are helpful in production of antibiotics (Ferrer *et al*, 2004).
4. These hyperthermophiles are used as biomarker to understand the metabolism and properties of persistent pollutant in the environment because they have wide range of tolerance and high rate of DNA stability at extreme conditions (Grogan, 1998).
5. The hyperthermal cellulose and hemicelluloses obtained from hyperthermophiles is been used in mimic industrial processes due to their high potential of stability at extreme temperature that are helpful for degradation of cellulose and hemicelluloses (Hongpattaraker, 2002).
6. The enzyme such Taq DNA polymerases obtained from *Thermus aquaticus* organism are widely been used in polymerase chain reaction and are widely been used in forensic genetic fingerprinting (Kampmann, and Stock, 2004).
7. They are used for denaturing toxic organic solvent, proteolytic enzymes and detergents due to their ability to enhance their activity in the presence of protein denaturants (Mai *et al.*, 1998).
8. HMf an nucleo protein complex obtained from hyperthermophile *Methanothermus fervidus* is under investigation for treatment of sickle cell diseases.
9. A eukaryotic homologue of the myc oncogene product from archae has been used to screen the sera of cancer patients (Maloney, 2003).
10. A recombinant strain of Deinococcus was capable of degrading organopollutants in radioactive mixed waste environment (Satyanarayana *et al.*, 2005).
11. Some of the hyperthermophilic sulphur oxidizing microorganisms was used for treatment of bioleaching, coal and waste desulfurization process.

Conclusion

Being the pioneer of our planet hyperthermophiles were now opening new realm of possibilities for the study of extra-terrestrial life by supporting in understanding microbial biodiversity and environmental conditions of extra terrestrial microorganisms. The current need is to emphasize more global research programs to understand their importance in evolution, possibilities of developing cost effective eco-friendly remediation processes and economical utility for humanity. For this very innovative purpose, we have to enhance their properties and use them according to our need either for removal of persistent pollutants, toxic chemicals, biohazards and radioactive wastes or in solving the energy crisis, making of novel compounds to cure deadly diseases such as Parkinsonôs, Alzheimerôs, AIDS, Cancer etc. and to understand life and environmental condition within this planet and beyond this planet.

Acknowledgment

The authors are grateful to the Prof. G. C. Pandey, Head, Depatrment of Environmental Sciences and other authorities of Dr. R. M. L. Avadh University, Faizabad, U.P., India were highly thankful for rendering their support and help for the completion of this work.

References:

Andrade M. M. C. A., Pereira N., Antranikian G., 1999. Extremely thermophilic microorganisms and their polymer-hidrolytic enzymes. *Revised Microbiology* 30, 287-298.

Atomi H., 2005. Recent progress towards the application of hyperthermophile and their enzymes. *Current Opinion of Chemical Biology* 9, 166-173.

Baghel S. V., Singh J., Gopal K., 2003. Antimicrobial resistance among enteric bacteria, isolated from runoff of the Gangotri glacier, western Himalaya. *Indian Journal of Environmental Biology* 24, 349-356.

Ferrer M., Chernikova T. N., Timmis K. T., Golyshin P. N., 2004. Expression of a Temperature Sensitive Esterase in a Novel Shaperone Based *Escherichia coli* Strain. *Applied Environmental Microbiology* 70, 4499-4504.

Grogan D. W., 1998. Hyperthermophiles and the problem of DNA instability. *Journal of Molecular Microbiology* 28, 1043-1050.

Hendry P., 2006. Extremophiles, Thereôs More to Life. *Environmental Chemistry* 3, 75-76

Hongpattaraker T., 2002. Hyperthermophiles Cellulolytic and Hemicellulolytic Enzymes and their Biotechnological Applications. *Journal of Science and Technology.* 24, 481-491.

Kampmann M., Stock D., 2004. Reverse gyrase has heat protective DNA chaperone activity independent of super coiling. *Nucleic Acid Research*. 32, 3537-3545.

Mai V. Q., Chen X., Hong R., Huang L., 1998. Small Abundant DNA Binding Proteins from the Thermoacidophilic Archaeon Sulfolobus shibatae Constrain Negative DNA Super coils. *Journal of Bacteriology* 180, 2560-2563.

Maloney S., 2003. Extremophiles Bioprospecting for antimicrobial; International Medical Press. On line from: http://www.mari_biotech.nstt.gov.cnMirrorResources/1895/extremophiles.cfm.htm-17K.

Robinson H., Gao Y. G., McCrary B. S., Edmondson S. P., Shriver J. W., Wang, A. H., 1998. The Hyperthermophiles Chromosomal Protein Sac-7d Sharply Kinks DNA. *Nature* 392, 202-205.

Satyanarayana T., Raghukumar C., Shivaji S., 2005. Extremophilic microbes: Diversity and perspective. *Current Science* 89, 78-90.

Tachibana Y., Kumramura A., Shirasaka N., Suzuki Y., Yamamoto T., Fujiwara S., Takagi M., Imanaka T., 1999. Purification and Characterization of an Extremely Thermostable Cyclomaltodextrin Glucanotransferase from a Newly Isolated Hyperthermophile Archeacon, a Thermococcus sp. *Applied Environmental Microbiology* 65, 1991-1997.