Environment Conservation Journal 12(1&2) 121-124, 2011
(ISSN 0972-3099) Abstracted and Indexed

Geothermal spring sites as excellent reservoir of novel microorganisms

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Received: 28.12.2010 **Accepted: 16.03.2011**

Abstract

Geothermal sites at the earth's surface are known to be present both on land and deep inside the sea. Thermophilic microorganisms are the only life forms that can survive under such harsh conditions. These extremophiles can resist the high temperature prevalent at a geothermal site for their capability to produce a variety of thermotolerant enzymes. Many of these enzymes are known to have applicability in a variety of industrial processes. The last three decades have witnessed the spectacular growth of industries producing thermostable enzymes either employing the pure strain or a recombinant one. In addition to that thermophilic microorganisms have been a source of other important metabolites. The evaluation of microbial wealth of every such site is therefore, a need of the hour.

Keywords: *Geothermal sites, Microorganisms, Enzymes*

Introduction

Greek words *geo* (earth) and *therme* (heat) defines the hot environment formed due to heat within the earth as 'geothermal' sites. Geothermal springs are biotopes with controlled environmental conditions round the year offering excellent conditions for the growth of thermophilic microorganisms. The diversity of such tiny life forms is a thrust area which is drawing attention of the researchers worldwide. A considerable temperature gradient exists inside the earth which is found to be quite high in certain geographical locations. The rainwater and snowmelt continue to seep underground owing to cracks and deep subterranean faults. As this water is heated by the hot rock a geothermal reservoir is formed beneath the earth. This hot water, through the openings at the earth's surface in the nearby region, forms geothermal springs, geysers or fumaroles (Bhardwaj and Tiwari, 2009).

A variety of flora and fauna is known to exist at the high temperature sites (Table-1). However, it is interesting to note that except prokaryotic organisms none of them is adapted for optimum growth at elevated temperature. Thermophilic microorganisms are capable to do so because of their ability to produce biocatalysts active at high temperature. Generally enzymes, being proteins, begin to denature at a temperature above 40 °C, and are completely inactive beyond 50-60 °C. However, thermotolerant enzymes have specific protein motiffs rendering the overall structure of its proteinaceous part unaffected at higher temperature. Some thermophilic enzymes are known to maintain at least half of their specific activities at temperature as high as 90 °C or above or in rare cases even higher. In other cases, enzymes might partially denature at high temperatures but have adaptive systems that allow them to renature into a functional form once removed from such extreme conditions.

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Microbial wealth of thermal springs

Microorganisms that can tolerate high temperatures are commonly found in hot springs or deep-sea thermal vents and are typically part of the group of Archaea. Although the Archaea might not grow fast enough or produce enough enzyme under "normal" conditions to make their harvest practical, their enzymes do have many potential applications in a wide variety of industrial processes where extreme conditions are required. Therefore, once the gene for potentially useful thermophilic enzyme has been identified, cloning techniques can be applied to express the enzyme under the control of a strong promoter, in a fast-growing organism that has been proven to show robust growth in large-scale fermentation systems. The heat stable enzymes from thermophilic and hyperthermophilic microorganisms have unlimited potential in biotechnological applications. The heat stable enzymes from the extremely thermophilic and hyperthermophilic microorganisms, virtually due to their unlimited potential in biotechnological applications are expected to fill the gap between biological and chemical processes (Leuschner and Antranikian, 1995). The extremophiles thus hold the promise of providing such enzymes which could be effective at high temperatures in industrial processes. Proteases, lipases, amylases and other hydrolases which are active at high temperature would be boon in food processing as fats could be hydrolysed, proteins digested and fibres modified enzymatically to make food more palatable and healthful (Sony and Sandhu, 1999).

Some of the previous work related to the isolation and characterization of microbial wealth of thermal springs across the globe is summarized in Table-2. The production of hydrolytic industrial enzymes (*e.g*. Protease, lipase, amylase, pullulanase, xylanase, pectinase, cellulase, lactase *etc*.) for the manufacturing of various valuable products has shown a spectacular rise during the last three decades. In 1983, the estimated sale of all industrial enzymes worldwide was estimated as US $ 1 billion. In 2009, the whole market for industrial enzymes has gone to be in the range of US $ 2.4 billion.

Table-1: Temperature range for the growth of various groups of organisms (Brock, 1978)

Organisms	Upper temperature limit
Animals	
Fish and other aquatic vertebrate	38 ^{0}C
Insects	45-50 ^{0}C
Crustaceans	49-50 ^{0}C
Plants	
Vascular plants	45 ^{0}C
Mosses	50 ^{0}C
Eukaryotic microorganisms	
Protozoa	56 ^{0}C
Algae	55-60 ^{0}C
Fungi	60-62 ^{0}C
Bacteria	
Cyanobacteria	70-73 ^{0}C
Photosynthetic bacteria	70-73 ^{0}C
Chemilthotrophic bacteria	90 ^{0}C
Heterotrophic bacteria	90 ^{0}C

Future Prospectus

There are some definite advantages in screening for wild type microbial genes among nature's own diversity. The gene expressed in nature represents proteins which presumably, through the evolutionary process has been undergoing hard and long selection pressure. The pressure thus exerted drives the gene evolution towards enzyme production which is most fit for solving interaction with the substrate needed for a given organism in an ecological niche in which it is adapted to inhabit. Careful selection of the taxonomic group and the ecological niche to screen, as compared to the industrial process conditions under which these metabolites should work gives optimal chances for discovery of novel microorganisms capable of producing them. Hot springs provide such opportunity in terms of wealth of biotechnologically important microflora.

Table -2: Microorganisms isolated from thermal springs having industrial utility

Thermal spring location	Microorganism	Usefulness/properties	References
Assam, India	*Brevibacillus laterosporus* BPM3	Biocontrol agent maximum growth and antagonistic activity at 30 °C, pH 8.5	---------------------
Siberian hot spring	*Crenarchaeota* sp.	Ammonia oxidizing, highly active at 0.14 and 0.79 mM ammonium	Hatzenpichler *et al*. (2008)
Iceland	*Thermoanaerobacter thermohydrosulfuricus*	Hydrogen and ethanol production Active at 50-78 ^{0}C	Koskinen *et al*. (2008)
Jae Sawn hot spring, Thailand	Recombinant *E.coli*	Lipase active 50-70 ^{0}C and pH (7-10)	Tieawongsaroj *et al*. (2008)
China	*Silanimonas lenta* and *Schlegelella aquatica*	Lipase producer, active at high temperature and alkaline conditions	Lee *et al*. (2005) and Chou *et al*. (2006)
Himachal Pradesh, India	*Bacillus* sp. J33	Thermostable lipase, heat stability increased after immobilization	Nawani *et al*. (2006)
Atri, Taptapani and Deuljhari, Orissa	*Bacillus* sp. *Pseudomonas* sp.	Heat stable lipase activity at 60^{0}C	Rath (1999)
Egyptian thermal spring	*Bacteria*	Thermostable cellulose production	Ibrahim and EI-diwany (2007)
Russia	*Caldicellulosiruptor kronotskyensis* and *Caldicellulosiruptor hydrothermalis*	NA	Miroshnichenko *et al*. (2008)
Korea	*Chlamydomonas taiwanensis*	Thermostable Amylase production	Chen *et al*. (2005)
Manikaran, Himachal	*Bacillus* sp.APR-4	Thermostable protease	Kumar *et al*. (2002)

Exploration of areas with extreme environments and the isolation and investigations on the associated microbial wealth for biotechnological applications are of great significance, both for basic and applied research within the country. In the whole world several attempts have been made by scientists in this direction but in India still a lot more attention has to be paid. The thermal springs of Uttarakhand are almost untouched for any such exploration.

Therefore, it seems to be of utmost importance to study the microbial diversity of these geothermal sites for their functional attributes. Further, novel techniques of metagenomic are expected to bring forth the hidden gold in the form of novel genes producing important metabolites from the uncultivated microbial world of such geographically distinct region.

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