Journal of Harbin Engineering University
ISSN: 1006-7043
Vol 44 No. 9
September 2023

Metagenomic Study for the Diversity Analysis of Extremophiles

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ABSTRACT
Microbial communities are the most abundant entities found in the Earth's biosphere. Due the limitations in culture techniques it is difficult to identify the microorganisms in the biosphere. Many organisms cannot survive in normal environment, extremophilic microorganisms surviving and thriving in extreme temperature like heat, cold, salinity, pH, pressure and radiation. These extreme environments are too harsh for normal life to exist. To analyze the microbial communities in these environments, a non-culture-based approach, called "metagenomics", was developed to provide the extensive information. The characterization of an organism in terms of molecular phylogenetic techniques based on 16S rRNA gene sequence comparisons have been employed, more recently high throughput pyrosequencing technology has been used to identify the uncultured microbes in the extreme environment. Therefore, the development of next generation sequencing technology allows metagenomic library to be rapidly constructed and sequenced, these provides a more comprehensive view of microbial diversity.
Keywords: Extremophiles, metagenomics, 16S rRNA, Microbial diversity, Hot spring.

INTRODUCTION:
The earth is rich in biological material that has not yet been explored. The world's every part is surrounded by the huge diversity of enormous and differential microbes. Microbes are living things that are too small to see with the naked eye generally smaller than about 0.2 mm. Microbiologists have specific names for the various types of microbes, which include bacteria, archaea, viruses, and small eukaryotes and fungi. It is widely accepted that the genomes of microorganisms represent the major reservoir of genetic diversity on earth (Ferrer *et al.*, 2009; Whitman *et al.*, 1998). Due to their long existence microorganism metabolism is much more diverse and also they involve in various biogeochemical cycles of the biosphere that are essential for life on earth. Microorganisms are mostly found in every habitat on Earth, such as water, soil, air, acidic hot springs, glacial ice, highly polluted environments, and deep in the Earth's crust.
Extremophiles are microorganisms able to grow optimally in extreme environments of temperature, pH, pressure, and salinity (Mukhtar *et al.,* 2022). This feature requires the stabilization of all cellular components, so that the functionality is maintained under conditions that would be harmful for most non- extremophile molecules. Now the Microbiologist using the comparative ribosomal RNA sequencing technology to identify the microbial population of the extreme environments. Extremophiles often require specific growth conditions that are difficult to achieve in a laboratory. Furthermore, it is estimated that less than 1% of all microorganisms are able to grow in culture, by using the metagenomic technology, it provide the way to obtain gene sequences and explore these organisms. In fact, the recent advent and application of high throughput next generation sequencing methods and computational analysis have enabled the discovery of novel molecules with biotechnological interest in these otherwise hidden organisms. As metagenomic tools become more accessible to the research community, allowing the investigation of microorganisms that cannot be cultured in the laboratory, exciting aspects of extremophilic biology are now being revealed (Cardoso *et al.*, 2010; 2011).

Life in extreme environments has been studied intensively focusing attention on the diversity of organisms and molecular and regulatory mechanisms involved. The proteins, enzymes and other biomolecules these were obtained from extremophiles play an important role in the field of biotechnology. This field of research has also attracted attention because of its impact on the possible existence of life on other planets (Satyanarayana *et al.*, 2005). Microorganisms that

are extensively present in nature, metagenomics involves the isolation of total DNA from the environmental sample and then cloned in a appropriate vector(Bacterial artificial chromosome) and therefore the genes that are expressed must be introduced in a suitable host. On the basis of 16S rRNA gene sequence analysis, the metagenomic techniques providing the huge amount of phylogenetic data and also gave the genetic information of those microbes that are not cultured in the laboratory conditions.

Microbial diversity present in Extreme Environment

The most important extreme environments found in nature and typical microbial groups or species that have been observed in them are listed in Table 1.1. The most important factors for the growth of extremophilic microorganisms are high or low temperature, high or low pH, and high salinity. The resulting environments, based on the elevated or low conditions, are then qualified as thermophilic, psychrophilic, alkalophilic, acidophilic and halophilic, respectively.

Table 1.1 Characteristics of extreme environments in which microorganisms can grow (modified from Prescott *et al*., 1999).

Stress	**Environmental conditions**	**Ecosystem**	**Microorganisms observed**	**References**
Temperature	-2.5 - 0°C	Polar regions	Flavobacterium Cryoconite, Proteobacteria, Bacteroidetes, Cyanobacteria Actinobacteria	Shi T et al. (1997) Arwyn Edwards et al.(2013) Carola Simon et al.(2009)
	0 - 4°C	Deep marine trenches	Pseudomonas	Ravenschlag K et al.(2001)
	65 - 95°C	Terrestrial hot springs Thermal spring	*Thermus sp.* Aquificae, Dictyoglomi, Eryarchaeota, Korarchaeota, Thermodesulfo bacteria, Firmicutes Green sulfur bacteria, Heliobacteria, Green non sulfur bacteria Stenotrophomonas, Aquaspirillum, Zavarzinella, Haliscomeno	Kristjansson JK et al.(1995) Bernd Wemheuer et al.(2013) Hanan I et al.(2010)

			bacteria, Rheinheimera, Tepidomonas *Nanoarchaeota Synechococcus*	Memory Tekere et al.(2011) Stetter et al.,(2013) Bhaya et al.,(2007)
	>100 - 121°C	Submarine vents	*Thermococcus barophilus*	Marteinsson VT et al.(1999)
Salt	> 6%	Salt brines, salterns	*Halobacterium sp.* *Salinibacter ruber* *Haloquadratum walsbyi* Haloalkaliphilic bacteria	Sleator RD & Hill C (2002) Alio,(2006) Rohit Ghai et al.(2011) S. P. Singh et al.(2010)
pH	pH 3 or lower	Sulfide-rich geothermal zones Acid mine drainage Sulphur-oxidising biofilm	Thiobacillus Leptospirillum, Sulfobacillus, Acidomicrobium, Ferroplasma acidarmanus, Thermoplasmatales Acidithiobacillus thiooxidans	Kristjansson JK et al.(1995) Jo Handelsman(2004) Daniel S Jones et al.(2011)
	pH 10 or above	Soda lakes	Bacillus sp.	Rees HC et al.(2004)
Pressure	500 - 1034 atm	Deep marine trenches	*Moritella yayanosii*	Nogi Y & Kato C (1999)
Radiation	3 - 5 Mrad	Nuclear power plants	*Deinococcus radiodurans*	Rainey FA et al.(1997)

1. Life forms survive at different temperature environment

The classification of living organisms based on their relation to temperature has always been considered as the most basic element of biological systematic (Kristjansson and Hreggvidsson, 1995). Figure 1.1 shows the optimum growth temperature (T_{opt}), living organisms can be grouped into four categories e.g. psychrophiles that have a T_{opt} below 20^0C, mesophiles that grow optimally between 20^0C, thermophiles that grow on 45 to 55^0C and hyperthermophiles on 105^0C (Madigan *et al.*, 1997).

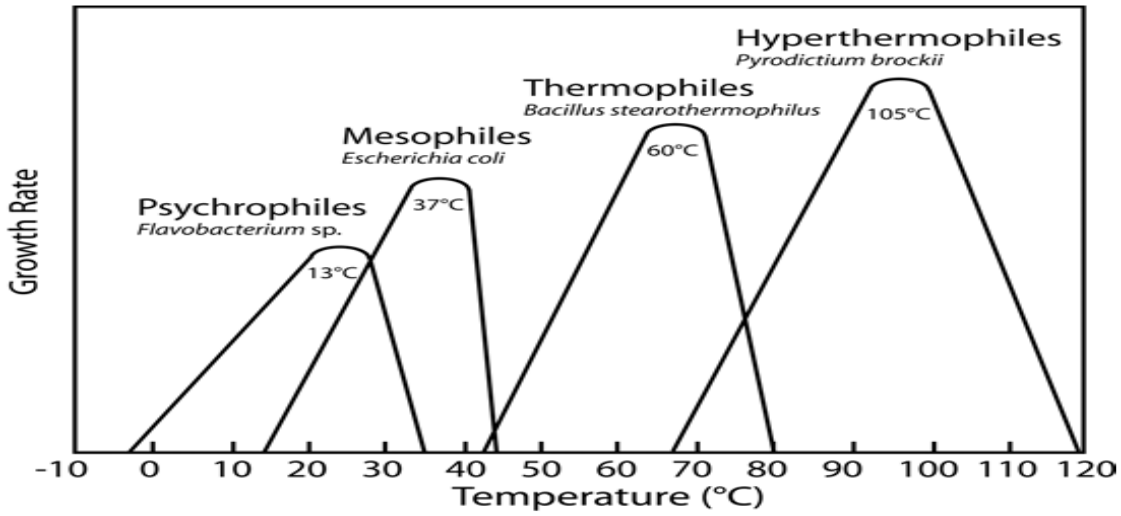

Fig. 1.1 Relation of temperature and growth rates for a typical psychrophilic, mesophilic, thermophilic and hyperthermophilic microorganism. The respective optimal growth temperatures T_{opt} are indicated on the graph.

1.1 High temperature field

High-temperature environments are generally found associated with volcanic activity, hydrothermal vents, hot springs. The most important biotopes are terrestrial geothermal fields, with alkaline freshwater hot springs and solfatara, and marine environments with coastal, shallow and deep hydrothermal systems. Hot environments display a complete range of pH, from acid to alkaline, depending on temperature, water availability, and gases and ion concentration (Kristjansson and Hreggvidsson, 1995).

Volcanic zones

Within the active volcanic zones, the temperature of water reaches upto 150 to 450^0C at the depth of 600 to 4000 m and these volcanoes contains the heat source i.e magma chamber at a depth of 3 to 6 km and also constitute steam, volcanic gases (mainly N_2 , CO_2 ,H_2S and H_2)are emitted at the surface. On the surface, H_2S is oxidized chemically and biologically first to sulfur and then to sulfuric acid which acts as the buffering agent in the hot spring environment. Steam and water are collected in boreholes 1500 to 2000 m deep and temperatures are ranging between 50 to 130^0C. These environments have been minimally investigated (Takai and Horikoshi,1999) yet constitute one of the few opportunities to examine the deep subsurface thermophilic microbial ecosystems.

The prokaryotic diversity of two microbial communities derived from thermoacidophilic spring near the Mutnovsky Volcano and from a thermophilic spring in the Uzon Caldera. Environmental DNA was isolated for metagenomic analysis. The Mutnovsky hot spring was dominated by the Terrestrial Hot Spring Group, Kosmotoga, and Acidithiobacillus. The Uzon Caldera was dominated by uncultured members of the Miscellaneous Crenarchaeotic Group and Enterobacteriaceae. The remaining 16S rRNA gene sequences belonged to the *Aquificae, Dictyoglomi, Eryarchaeota, Korarchaeota, Thermodesulfobacteria, Firmicutes,* and some potential new phyla (Wemheuer *et al*., 2013).

Hot springs

Many hyperthermophilic archaea and bacteria have been isolated from hot spring environments.

A study of microbial diversity in hot spring samples showed that all the hot springs had similar temperatures (between 85^0C and 95^0C) and a pH value (7.8-8.9), but they differed markedly with regard to their overall microbial diversity (Thomas, Blank *et al.*, 2002). The microbial diversity was investigated at five major hot springs in Jordan (Ashounah, Waggas, Zara, Zarqa Ma'in and Afra springs) using both microbiological culture-based and molecular culture independent approaches. Out of 132 isolates, 125 isolates were gram positive rods, while the other seven isolates were gram positive coccobacilli. The bacterial growth at different high temperatures was determined, and revealed that 19 out of 132 isolates were able to grow at high temperatures of up to 75^0C. Culture-independent approaches using polymerase chain reaction (PCR) was used for the amplification of specific 16S rRNA sequences of Bacteria, Archaea, Green sulfur bacteria, Green nonsulfur bacteria, Heliobacteria, and methanogenic Archaea from metagenomic DNA extracted directly from water and mat samples from each thermal spring (Malkawi and Al-Omari ,2010).The study of extreme environments has considerable biotechnological potential; an example is the *Taq* DNA polymerase, purified from the hot springs bacterium *Thermus aquaticus.* The recent interest in biotechnology, coupled with the discovery of novel thermophiles, has prompted studies on the utilization of thermophiles and their enzymes for industrial purposes (kanokratana *et al.*, 2004). The diversity of Crenarchaeota was investigated in eight terrestrial hot springs (pH 2.8–7.7; temperature 44– 96^0C) located in Tengchong, China, using 16S rRNA gene phylogenetic analysis (Song *et al.*, 2010).

The first PCR-based studies of 16S rRNA genes, carried out on organisms from the hot springs of Yellowstone National Park (YNP), revealed an unexpectedly high diversity in contrast with the estimate from culture-dependent studies. This finding led to a revolution in the understanding of phylogenetic relations between Archaea and Bacteria (Barns *et al*., 1994, 1996). Phylogenetic diversity from these hot springs, *i.e.*, the archaeal phylum *Crenarchaeota* and the bacterial division *Aquaficales (*Barns *et al*., 1996; Meyer-Dombard *et al*., 2005), and even to the discovery of a new archaeal phylum classified as the *Korarchaeota (*Barns *et al.*, 1996).However, the also new archaeal phylum *Nanoarchaeota* remained undetectable by conventional PCR-based studies until it was discovered using non-traditional culturing techniques. It is represented by *N. equitants,* a nanosized hypertermophilic symbiont from a submarine hot vent, whose rRNA gene sequence is unique, even in the highly conserved regions used as primer targets for PCR (Huber *et al.*, 2002). The newly found *Nanoarchaeota* and *Korarchaeota* are members of the deepest branch-offs of the rRNA phylogenetic tree (Stetter *et al*., 2013). *Synechococcus*, an inhabitant of microbial mats in hot springs along thermal gradients where ecologically different subpopulations have been found by exhaustive metagenomic analysis (Bhaya *et al.*,2007).Temperature, as a shaping agent in a bacterial microbial mat of effluent channels from two hot springs in YNP, was analyzed by bar-coded pyrosequencing (Miller *et al.*, 2009) . This technique entails the introduction of different codes in the primers used with the different samples so as to assign a sample origin to each sequence retrieved after pyrosequencing (López-López *et al*., 2013).

The bacterial diversity of Siloam hot water spring was determined using 454 pyrosequencing of two 16S rRNA variable regions V1-3 and V4-7. Analysis of the community DNA revealed that the phyla Proteobacteria, Cyanobacteria, Bacteriodetes, Planctomycetes, Firmicutes, Chloroflexi and Verrucomicrobia were the most abundant. The bacterial diversity detectable and classifiable was greater when the V4-7 variable region was used compared to the V1-3 region. The most abundant bacteria genera detected with region V1-3 were; *Stenotrophomonas* (23.3%), *Aquaspirillum* (5.11%), *Zavarzinella* (2.73%), *Haliscomenobacteria* (1.25%), *Rheinheimera* (1.14%) and *Tepidomonas* (1.14%). All the other detectable genera were below 0.6%. Genera detected with region V4-7 from most abundant were; *Stenotrophomonas* (17.96%), *Zavarzinella* (5.81%), *Aquaspirillum* (4.75%), *Rheinheimera* (3.52%), *GPI* (1.41%), *Gemmata* (1.41%) and *Syntrophobacter* (1.06%). All the other genera detected were below 0.7%. Siloam is one of the hottest thermal springs in South Africa (63^0C), the water has a pH of 9.5 and is relatively high in fluoride and bromide; it is possible that the physicochemical properties could have some influence on the diversity of bacteria. This was the first phylogenetic analysis of a South African thermal spring bacterial community (Tekere *et al.*, 2011)

Hydrothermal vents

Hydrothermal vents occur over a wide depth range, from intertidal to the abyss (Tarasov *et al*., 2005). Since the discovery the deep-sea hydrothermal vent in the 1970s, these environments have been regarded as one of the main habitats for thermophiles. Indeed, many thermophilic archaeal and bacterial species have been isolated from deep-sea vent sites. Molecular analysis of the hydrothermal vent communities has revealed phylotypes that have not yet been found in cultivated or in 16S rRNA clone libraries.

1.2 Low temperature field

The largest part of the Earth's biosphere is experiences low temperatures in which the oceans covers 71% of the earth surface have temperature of 5^0C and the depth of the ocean have temperature between 1^0C to 4^0C. Therefore, the polar regions covers the 14% of the earth surface, which are permanently frozen. These environments, which are dominant on Earth, are favorable to psychrophiles able to grow at any cold temperature at which water is still liquid (Madigan *et al*., 1997). The psychrophiles that permanently live on the temperature at 0^0C. The temperature of the atmosphere is $<5^0$C at the altitude of >4000 m, therefore when the altitude increases suddenly the decrease in temperature and it reaches upto below -40^0C. According to (Morita, 2000) cold environments can be divided into two categories: psychrophilic (permanently cold) and psychrotrophic (seasonally cold or where temperature fluxes into mesophilic range) environments.

Glaciers and polar ice caps

Glaciers are generally the huge masses of snow, recrystallized ice and rock debris that accumulate in great quantities and begin to flow outwards and downwards under the pressure of their own weight. Glaciers can be defined as simple, relatively closed ecosystems sustained by primary producers (e.g., photosynthetic bacteria and algae) in the snow and ice (Choudhari *et al.*, 2013).

Cryoconite is a microbe–mineral aggregate which darkens the ice surface of alpine glaciers. Microbial process and marker gene PCR-dependent measurements reveal active and diverse cryoconite microbial communities on polar glaciers. The metagenome revealed a bacterially-dominated community, with Proteobacteria (62% of bacterialassigned contigs) and Bacteroidetes (14%) considerably more abundant than Cyanobacteria (2.5%). Cryoconite holes are thought to represent 'ice-cold hot-spots of microbial diversity and activity (Edwards *et al.*, 2012) at the surface of Earth's largest freshwater biome, namely glaciers and ice-sheets (Hodson *et al.*, 2008; Shiklomanov, 1993).

Pyrosequencing analyzed the phylogenetic composition and metabolic potential of the microbial assemblage present in glacier ice of the Northern Schneeferner, which is the largest and highest glacier of the five glaciers located in the German Alps. In this study, firstly DNA was isolated from glacial ice , pyrosequencing of this DNA yielded 1,076,539 reads (239.7 Mbp). The phylogenetic composition of the prokaryotic community was assessed by evaluation of a pyrosequencing-derived data set and sequencing of 16S rRNA genes. The Proteobacteria (mainly Betaproteobacteria), Bacteroidetes, and Actinobacteria were the predominant phylogenetic groups. In addition, isolation of psychrophilic microorganisms was performed, and 13 different bacterial isolates were recovered (Simon *et al.*, 2009).

Cold environments are large reservoirs of microbial life. The study employed 16S rRNA gene amplicon metagenomic sequencing to survey the prokaryotic microbiota on Alaskan glacial ice, revealing a rich and diverse microbial community of some 2,500 species of bacteria and archaea (Choudhari *et al.*, 2013).Using mothur (Schloss *et al*., 2009) with the Ribosomal Database Project (RDP) (Cole *et al.*, 2009) and SILVA (Pruesse *et al.*, 2007) databases, they performed an initial phylogenetic analysis and identified 2,459 operational taxonomic units (OTUs) at a 97% identity cutoff (species level). Proteobacteria (~40%) was the most abundant phylum of bacteria. Other sequences were classified as follows: Bacteroidetes (~22%), Firmicutes (~12%), Actinobacteria (~9%), Cyanobacteria (~5%), Acidobacteria (~3%), Verrucomicrobia (~3%), and Planctomycetes (~2%). An early report 45 years ago (Kol, 1968) listed 354 algal and cyanobacterial species, 77 fungal species, and 35 bacterial species that occur in snow. A recent metagenomic study of glacial ice in the German Alps (Simon *et al*., 2009) identified 72 bacterial OTUs at a 97% identity cutoff (and 108 OTUs at a 99% cutoff).

1.3 Hypersaline environment

Hypersaline environments, which contain almost saturating concentrations of sodium chloride, are often dominated by archaeal and bacterial halophilic microorganisms (Benlloch *et al.*, 2002). However, the overall diversity in extreme hypersaline environments is generally low. Besides the dominance of archaea, a new bacterium, *Salinibacter ruber*, that is an important component of the halophilic microbial communities. *S. ruber* can comprise up to 25% of the total prokaryotic community in hypersaline environments (Alio, 2006).

The two hypersaline saltern ponds near Alicante (Spain),one of intermediate salinity (19%) and a NaCl saturated crystallizer pond (37%) using pyrosequencing. The analyses of these metagenomes (nearly 784 Mb) reaffirmed the vast dominance of *Haloquadratum walsbyi* (Ghai *et al.*, 2011).

Haloalkaliphilic bacteria, a group of organisms with twin extremities of pH and salinity, have traditionally been investigated from variety of habitats (Singh *et al.*, 2010). Haloalkaliphilic bacteria have largely been explored and studied from the concentrated hypersaline environments; Soda Lake, Solar Saltern, Salt brines, Carbonate springs and Dead Sea (Singh, 2006) . They were working on haloalkaliphilic bacteria over the last 15 years and have indicated their wide occurrence in moderately saline environment of coastal region of Gujarat in India. Large number of haloalkaliphilic bacterial strains depicted wide diversity, as reflected through microbiological examinations, biochemical characteristics and molecular approaches (Dodia *et al.*, 2008; Gupta *et al.*, 2005; Nowlan *et al.*, 2006;). As an extension of our on-going research on haloalkaliphilic bacteria form coastal Gujarat (Dodia *et al*., 2008 ; Gupta *et al*., 2005; Manikandan *et al.*, 2009; Singh, 2006; Thumar *et al.*, 2007),they have also adapted metagenomic approaches to get insight into the non-cultivable microbes to trap the potential of the majority of the bacteria from the saline habitats(Joshi *et al.*, 2009).

The brines of saltern crystallizer ponds worldwide are colored pink-red by *Archaea* (*Haloquadratum* and other representatives of the *Halobacteriales*), *Bacteria* (*Salinibacter*), and *Eucarya* (*Dunaliella salina*) (Yanhe *et al*., 2010).

1.4 Extreme pH environments

The overall microbial diversity in extreme pH environments is usually low compared with microbial diversity in non-extreme pH environments. Hydrogen sulfide (H_2S) is released in large amounts and oxidized chemically and biologically into sulfuric acid resulting into the decrease of the pH in the soil. On the other hand, there are few examples of highly alkaline biotopes in nature. Soda lakes in West-Africa, Tibet, China or California have pH values reaching 11 to 12 but also contain high concentrations of salts. Unlike natural hypersaline lakes and seas they are depleted in Ca^{2+} and Mg^{2+} ions which disappeared at the early stages of the creation of the lake by precipitation of carbonates (Grant WD, 1991).

The best example of such an analysis is the nearly complete sequencing of the metagenome of a community in acid drainage of the Richmond mine, which represents one of the most extreme environments on Earth. The microbial community forms a pink biofilm that floats on the surface of the mine water. The drainage water below the biofilm has a pH of between 0 and 1 and high levels of Fe, Zn, Cu, and As (317, 14, 4, and 2 mM, respectively). The community is dominated by a few bacterial genera, *Leptospirillum*, *Sulfobacillus*, and sometimes *Acidomicrobium*, and one archaeal species, *Ferroplasma acidarmanus*, and other members of its group, the *Thermoplasmatales* (Handelsman, 2004).

Snottites are extremely acidic (pH 0–1) biofilms, form on the walls and ceilings of hydrogen sulfide-rich caves. Using metagenomics, Snottites from the Frasassi cave system (Italy) are dominated (470% of cells) by *Acidithiobacillus thiooxidans* (Jones *et al.*, 2011).

1.5 Extreme pressure environment

The *Moritella yayanosii*, barophilic bacteria collected from world's deepest ocean floor in the Mariana Trench challenger deep at a depth of 10898 m (Nogi and Kato, 1999). Barophilic bacteria are characterized by enchanced growth at pressure 500 - 1034 atm. The report by (Yayanos *et al*., 1979), numerous deep-sea barophilic bacteria strains have been isolated and characterized in an effort to understand the interaction between the deep-sea environment and its microbial inhabitants (Kato *et al*., 1995,1996,1998). Yayanos et al. (1979) were

successful in collecting an amphipod (*Hirondella gigas*) at the depth of 10476m.

1.6 Extreme radiation environment

Extreme emissions of radiation are mostly found in man-made nuclear facilities. Very few microbes have been identified originating from the pools in which fissile material is stored. Among these, *Deinococcus radiodurans* (Rainey *et al.*, 1997) can survive as much as 3 to 5 Mrad when a lethal dose for humans is 0.0001 Mrad. Recent research indicates that its ability to resist radioactivity may results from the presence of multiple copies of the chromosome and the ability to repair severely damaged DNA (Prescott *et al.*, 1999).

Investigation of microbial diversity in several habitats

Molecular phylogenetic techniques based on 16S rRNA gene sequence comparisons have been employed to investigate microbial diversity in several habitats, terrestrial hot spring microbial communities were among such habitats to be surveyed with this technology (kanokratana *et al.*, 2004; Ward *et al.*, 2002). The application of these techniques to study natural microbiotas in hot springs without the traditional requirement for cultivation has allowed the identification and study of many previously undetected organisms (Hobel *et al.*, 2005; kanokratana *et al.*, 2004; Skirnisdottir *et al*., 2000), since typically only a small fraction (<1%) of naturally occurring microorganisms is routinely cultivatable by standard techniques (Barns *et al*., 1996; Handelsman, 2004; Skirnisdottir *et al*., 2000). The 16S rRNA molecule are universally conserved and suitable for phylogenetic studies of distantly related organisms. The characterization of an organism in terms of its phylotype requires only a 16S rRNA genes or gene fragments can be selectively amplified by PCR from complex DNA mixtures obtained directly from the environment. A set of oligonucleotide probes universal for all Bacteria or Archaea or targeted at specific taxonomic levels (phylum, families, genus) are used to amplify specifically the genes. As a final , a phylogenetic tree is created, gathering all information regarding the relationship between the newly obtained sequences and the reference sequences. Recently, high-throughput pyrosequencing technology has been applied for the metagenomic characterization of environmental microbial communities (Biddle *et al.*, 2008; Dinsdale *et al*., 2008). Pyrosequencing analyzed the phylogenetic composition and metabolic potential of the microbial assemblage present in extreme environment.

CONCLUSION

In this review microbial ecology examine the diversity of life forms found in almost in any extreme conditions. Using a metagenomics approach several studies have provided a wealth of information on microbial diversity in extreme environmental conditions. Metagenomics can provide the tools to balance the abundance of knowledge attained from culturing, with an understanding of the uncultured majority of microbial life. Metagenomics may further increase our understanding of many of the exotic and familiar habitats that are attracting the attention of microbial ecologists. It includes deep sea thermal vents, acidic hot springs, permafrost, temperate, desert, cold soils, Antarctic frozen lakes.

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