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Archaeal Diversity and Their Biotechnological Potential

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1. Introduction

The curiosity of identifying, grouping, and naming organisms according to their established natural relationship has been the subject of a great interest since ancient times. Today, instead of the traditional rank-based biological classification, phylogenetic systematics, which aims at postulating phylogenetic trees rather than focusing on what taxa to delimit, has been used commonly. Carl Woese is the one who first realized that the ribosome, the ubiquitous molecular structure that conducts protein synthesis, offers a way to investigate systematically the relationships between all forms of life. Woese's approach was to determine the sequences of the RNAs that make up the ribosome, particularly the small subunit of ribosomal RNA (rRNA) (Woese et al., 1990). Comparisons of nucleotide sequences of ribosomal genes from different organisms allowed understanding of the evolutionary relationships between the organisms: the higher the similarity or difference between the rRNA sequences, the more or less closely related the organisms are. Instead of the commonly accepted subdivision of living organisms into the five kingdoms: *Monera*, *Protista*, *Fungi*, *Animalia*, and *Plantae* (Whittaker, 1969), Woese and his colleagues proposed subdivision into three higher taxa: *Archaea*, *Bacteria*, and *Eukarya*, first, they called them primary kingdoms and then domains (Woese et al., 1990). The sequencing of rRNA genes became one of the main tools for the construction of phylogenetic backbone of microbial classification and today each new description of *Bacteria* and *Archaea* must be accompanied by the complete 16S rRNA sequence of the type strain (Ludwig & Klenk, 2001; Yarza et al. 2010). Although the 16S rDNA gene has been tremendously useful for establishing the molecular phylogeny of prokaryotes over the last three decades, it suffers from the same limits as any other single-gene phylogenetic approach does. The identification of microbial isolates by whole-cell mass spectrometry (WC-MS) is being recognized as one of the latest tools bringing a revolution in microbial diagnostics, with the potential of bringing to an end many of the time-consuming and man-power-intensive identification procedures that have been used for decades. Apart from applications of WC-MS in clinical diagnostics, other fields of microbiology also have adopted the technology with success. MALDI-TOF MS shows particular potential usefulness for applications in environmental microbiology, e.g., to rapidly reveal cryptic species in large batches of related isolates (Clermont et al., 2009; Welkera & Moore, 2011).

Majority of the living beings thrive in environments having physically and geochemically temperate conditions. The extreme environments found on the planet are generally inhabited by microorganisms, which belong to the archaeal and bacterial domains of life. Extreme environments comprise the sites including physical variables as -20°C to +113°C (like stratosphere and hydrothermal vents), ≤120 Mpa (for hydrostatic pressures in the deep sea), aw ≈ 0.6 (for the activity of water in salt lakes) and ≈ 0.5< pH < 11 (for acidic and alkaline biotopes) (Woese et al., 1990). Archaeal ecology is generally accepted as synonymous with extreme environments in the point of the human being view. Representatives of *Archaea*, however, occur everywhere: in samples from ocean water, ocean sediments, freshwater lakes, soil, solid gas hydrates, tidal flat sediments, plant roots, peatlands, petroleum-contaminated aquifers, human subgingival area, skin and gastrointestinal tract and as a simbiyont within the sponge (Cavicchioli et al., 2003; Mills et al., 2005; DeLong, 2005; Knittel et al. 2005; Fierer et al., 2007; Brochier-Armanet et al. 2008; Oxley et al. 2010; Kong, 2011).

The majority of extremophiles belongs to the *Archaea*, the third domain of the living organisms together with *Eukarya* and *Bacteria* as explained before (Woese et al., 1990). The *Archaea* are a prokaryotic domain known to be often associated with habitats of extreme temperature, salinity and pH, and their presence in constantly cold marine waters is also well documented (Karr et al., 2006). Archaeal 16S rRNA community analysis has demonstrated that novel groups of *Archaea* are also abundant in the open ocean, soil and freshwater ecosystems as well (Buckley et al., 1998; Falz et al., 1999). *Archaea* exist in a broad range of habitats, and as a major part of global ecosystems, may contribute up to 20% of Earth's biomass (DeLong & Pace, 2001). *Archaea*, the most recently recognized domain, contains cultivated members that span a fairly limited range of phenotypes, represented by extreme halophiles, Sulfate-reducers and sulfur-metabolizing thermophiles, and methanogens. The first-discovered *Archaea* were extremophiles, which can be divided into four main physiological groups. These are the halophiles, thermophiles, alkaliphiles, and acidophiles.

Organisms from the domain *Archaea* differ fundamentally from *Eukarya* and *Bacteria* in several genetic, biochemical, and structural properties. Archaeal species have been classified as an early-branching evolutionary offshoot of the domain *Bacteria* and have long been considered to represent a primitive form of life that thrives only in extreme environments such as hot springs, salt lakes, or submarine volcanic habitats. However, recent researches have shown that *Archaea* are more physiologically diverse and ecologically widespread than was previously thought. Like *Bacteria*, *Archaea* are commonly mesophilic, and some members are known to be closely associated with eukaryotic hosts, including humans. For instance, high numbers of methane-producing *Archaea* (methanogens) have been detected in the gastrointestinal tract, vagina, and oral cavity (Belay et al., 1990; Vianna et al., 2006) and recently non-methanogenic *Archaea* including members of the *Crenarchaeota*, *Thermococcales*, *Thermoplasmatales* and *Halobacteriaceae*, have been detected in human faeces (Oxley et al., 2010). They are now recognized as a component of human microbiota and it is subjects of debate wheather archaea are cause of any disease in human. Although it was shown that members of the domain *Archaea* are found in greater abundance in dental plaque from sites with periodontal disease than in plaque from non-diseased sites (Lepp et al., 2004), it is generally assumed that archaea are not a cause of human disease (Vianna et al., 2006). The

isolation of archaeal strains from root-canal samples by amplification of 16S rDNA were at first unsuccessful, however, a recent study has confirmed that members of the methanogenic archaea, similar to *Methanobrevibacter oralis*, can be detected in root canal samples (Vianna et al., 2006). Finally, the presence of archaea in root canals has been confirmed and this provides new insights into the polymicrobial communities in endodontic infections associated with clinical symptoms.

Archaea, one of the three domains of life on Earth, is predominantly composed of two major phyla: the *Euryarchaeota*, the *Crenarchaeota*. In addition to these two major phyla, the *Korarchaeota* (Elkins, 2008), the *Nanoarchaeota* (Huber et al., 2002), the *Thaumarchaeota* (Brochier-Armanet et al., 2008) and the *Aigarchaeota* (Nunoura et al., 2011) have been proposed to be potential phylum-level taxonomic groups within the *Archaea*; however, the establishment of these phyla is still controversial (Takai & Nakamura, 2011). This division, based on small-subunit rRNA phylogeny, is also strongly strengthened by comparative genomics and phenotypic characteristics. The first archaeal genome was sequenced in 1996 and so far 52 genomes of *Archaea* have been sequenced. The cultured *Crenarchaeota* are composed of four orders: *Caldisphaerales*, *Desulfurococcales*, *Sulfolobales* and *Thermoproteales* (Chaban et al., 2006). *Euryarchaeota* are composed of nine orders *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales* (Balch et al., 1979), *Methanosarcinales* (Boone et al., 2001), *Halobacteriales* (Grant & Larsen 1989), *Thermoplasmatales* (Reysenbach, 2001), *Thermococcales* (Zillig et al., 1987), *Archaeoglobales* and *Methanopyrales* (Huber & Stetter, 2001).

The metabolic diversity of archaea is quite similar to bacteria in many aspects. Except for methanogenesis, all metabolic pathways discovered in archaea also exist among bacteria. Archaeal species can be either heterotrophs or autotrophs and use a large variety of electron donors and acceptors. Photosynthesis based on chlorophyll has not been found in *Archaea*, whereas photosynthesis based on bacteriorhodopsin, once believed unique to halophilic archaea, has been recently found in planktonic bacteria as well (Forterre, 2002). Beside their specific rRNA, archaea can be distinguished from bacteria by the nature of their membrane glycerolipids that are ethers of glycerol and isoprenol, whereas bacterial and eukaryal lipids are characterized as esters of glycerol and fatty acids (Kates, 1993). Archaeal glycerolipids are also 'reverse lipids', since the enantiomeric configuration of their glycerophosphate backbone is the mirror image of the configuration found in bacterial and eukaryal lipids. Another difference between *Archaea* and *Bacteria* is the absence of murein in archaea, whereas this compound is present in the cell wall of most bacteria. *Archaea* exhibits a great diversity of cell envelopes (Kandler & Konig, 1998), most archaea have a simple S-layer of glycoproteins covering the cytoplasmic membrane, whereas a few of them (*Thermoplasmatales*) only have a cytoplasmic membrane containing glycoproteins. The difference between *Archaea* and *Bacteria* at the molecular level is exemplified by the resistance of archaea to most antibiotics active on bacteria. Early studies on the molecular biology of archaea have shown that this resistance was due indeed to critical differences in the antibiotic targets (Zillig, 1991).

Ammonia oxidation carried out by microorganisms has global importance in nitrogen cycling and is often thought to be driven only by ammonia-oxidizing bacteria; however, the recent finding of new ammonia-oxidizing organisms belonging to the archaeal domain challenges this notion. Two major microbial groups are now considered to be involved in ammonia oxidation. These are chemolithoautotrophic ammonia-oxidizing bacteria and

ammonia-oxidizing archaea. The first isolated ammonia-oxidizing archaeon , *Nitrosopumilus maritimus*, from a tropical marine aquarium tank (Konneke et al., 2005), was reported to include putative genes for all three subunits (*amoA*, *amoB*, and *amoC*) of ammonia monooxygenase that is the key enzyme responsible for ammonia oxidation. Ammonia-oxidizing archaea are determined to thrive in various habitats including marine and fresh waters, hot/thermal springs, soils, and wastewater treatment systems (Youa et al., 2009).

The fascinating ability of members of the *Archaea* to thrive in extremes of temperature, salt and pH as well as in other seemingly hostile niches has generated substantial interest in the molecular mechanisms responsible for mediating survival in the face of such environmental challenges. Despite the obvious potential of extremophilic archaea to yield many commercially appealing enzymes, thermostable DNA polymerases remain the only major class of molecule to have been effectively exploited in a wide range of PCR protocols.

2. Halophilic Archaea

Halophilic archaea (also called halobacteria or haloarchaea) are present in high abundance and are often the dominant prokaryotes in hypersaline environments on the Earth such as solar salterns, hypersaline lakes, the Dead Sea, hypersaline microbial mats and underground salt deposits (Oren, 2002). Most known halophiles are relatively easy to grow, and genera such as *Halobacterium*, *Haloferax*, and *Haloarcula* have become well known models for studies of the archaeal domain because they are much simpler to handle than methanogenic and hyperthermophilic archaea (Ma et al., 2010).

The haloarchaea are a monophyletic group including all known aerobic, obligate halophilic archaea. All are chemoorganotrophs; most utilize carbohydrates or amino acids and grow optimally between 3.4-4.5 M NaCl and generally require a minimum of 1.5 M NaCl for growth, some even grow well in saturated (>5 M) NaCl (Grant et al., 2001). In this respect, many haloarchaea appear ecologically equivalent in terms of resource and physiological requirements and therefore exhibit considerable overlap in their fundamental niches, defined as the combination of conditions and resources which allow a species to maintain a viable population (Begon et al., 1986). However, there are notable exceptions to this observation. For example, while most halophilic archaea exhibit optimal growth at near neutral pH, many are alkaliphilic and require at least pH 8.5 for growth (Grant et al., 2001). Recent molecular studies have revealed the presence of halophilic archaea in several low salinity environments (Elshahed et al., 2004). In addition, Purdy and colleagues (2004) isolated haloarchaea from a coastal salt marsh that exhibited optimal growth at 10% (1.7 M) NaCl but could grow slowly at 2.5% (0.43 M) NaCl. Microbial life has adapted to environments that combine high salt concentrations with extremely high pH values. Alkaline soda lakes in Africa, India, China, and elsewhere with pH values of 11 and higher and salt concentrations exceeding 300 g/l (5.1 M) are teeming with life (Oren, 2002). Hypersaline environments are found in a wide variety of aquatic and terrestrial ecosystems. These environments are inhabited by halotolerant microorganisms but also halophilic microorganisms ranging from moderate halophiles with higher growth rates in media containing between 0.5 M and 2.5 M NaCl to extreme halophiles with higher growth rates in media containing over 2.5 M NaCl (Ventosa et al., 1998). Aerobic, anaerobic, and facultative anaerobic microbes belonging to domains *Archaea* and *Bacteria* have been recovered from these extreme ecosystems, where they participate in overall organic matter oxidation (Oren, 2002; Moune et al., 2003; Hedi et al., 2009).

Hypersaline habitats are a kind of extreme environment dominated by halophilic archaea, which require a minimum of 9% (w/v) (1.5 M) NaCl for growth (Grant et al., 2001). In general, haloarchaeal strains require high salt concentration for growth and cell integrity. They, with some exceptions, lyse or lose viability in low salt concentrations or distilled water, and water sensitivity or lysis-resistence has been a key differentiation criterion between halococci and other haloarchaea (Grant et al., 2001). The most well known haloarchaea *Halobacterium salinarum*, for example, requires at least 2.5 M NaCl for growth and cells lose their morphological integrity instantaneously at less than 1 M NaCl (Kushner, 1964). Another representative, *Halococcus morrhuae* does not lyse in distilled water (Grant et al., 2001).

Over the last decade, the diversity of halophilic archaea in various hypersaline environments has been examined and more fully characterized. Included among these studies are naturally occurring salt lakes, hypersaline microbial mats and man-made solar salterns (Benlloch et al., 2001; Maturrano et al., 2006). In another study the microbiota in colonic mucosal biopsies from patients with inflammatory bowel disease, 16S rDNA sequences representing a phylogenetically rich diversity of halophilic archaea from the *Halobacteriaceae* were determined. The study revealed a multitude of undefined bacterial taxa and a low diversity of methanogenic archaea (Oxley et al., 2010). Representatives of *Archaea*, the third domain of life, were generally thought to be limited to environmental extremes of the earth. However, the discovery of archaeal 16S rRNA gene sequences in water, sediment and soil samples has called into question the idea of archaea as obligate extremophiles (Purdy et al., 2004).

As mentioned above based on the relationship with NaCl, the most salt requiring archaea are found in the order *Halobacteriales* and *Methanosarcinales*. They belong to the phylum *Euryarchaeota*; no halophilic representatives have yet been identified within the *Crenarchaeota* (Oren, 2008). Within the small subunit rRNA gene sequence-based tree of life there are three groups of prokaryotes that are both phylogenetically and physiologically coherent and consist entirely or almost entirely of halophiles. Within the *Euryarchaeota* the order *Halobacteriales* occurs with a single family, the *Halobacteriaceae* (Oren, 2002). The numbers of genera is reached to 35 (November 2011- Euzebylist) and *Salarchaeaum* is the last added extremely halophilic genus (Shimane et al., 2011). Most species of *Halobacteriaceae* are true extreme halophiles according to Kushner's definition (Kushner, 1978), however, *Halobacteriaceae* contains some species which can grow in low salinity for instance, *Haloferax sulfurifontis* (Elshahed et al., 2004), *Haladaptatus paucihalophilus* "the spesific epithet refers to low salt loving", (Savage et al., 2007) and *Halosarcina pallida* (Savage et al., 2008).

Diverse 16S rRNA gene sequences related to haloarchaea were recovered from tidal marine and salt marsh sediments, suggesting the existence of haloarchaea capable of growth at lower salt levels. In a recent study it was reported that two of three newly isolated genotypes had lower requirements for salt than previously cultured haloarchaea and were capable of slow growth at sea-water salinity (2.5% w/v NaCl). They reported the existence of archaea that could grow in non-extreme conditions and of a diverse community of haloarchaea existing in coastal salt marsh sediments and they concluded that the ecological range of these physiologically versatile prokaryotes is much wider than previously supposed (Purdy et al., 2004). Halophilic adaptation of organisms has been the subject of the great interest. Halophilic microorganisms have developed various biochemical strategies to adapt to high saline conditions, such as compatible solute synthesis to maintain cell

structure and function (Tehei, 2002). These solutes are clearly of industrial interest. Besides these metabolical and physiological features, halophilic microorganisms are known to play important roles in fermenting fish sauces and in transforming and degrading waste and organic pollutants in saline waste waters (Hedi et al., 2009). All halophilic archaea studied balance the high osmolarity of their environment by having an at least equimolar intracellular salt concentration, KCl instead of NaCl in well-energized cells (Sopa, 2006). It has been shown that typical haloarchaeal proteins differ from mesohalic proteins by having a high fraction of acidic residues and a reduced fraction of basic residues. The genome sequences have corroborated that result and shown that a theoretical 2D gel of a haloarchaeon differs remarkably from that of other organisms (Tebbe et al., 2005). The cytoplasmic membranes of halophilic archaea of the family *Halobacteriaceae* contain interesting ether lipids and often have retinal proteins (bacteriorhodopsin, halorhodopsin, and sensory rhodopsins). It is known that unsaturated ether lipids are far more common in the halophilic archaea than generally assumed. Such unsaturated diether lipids were earlier reported from the psychrotolerant haloarchaeon *Halorubrum lacusprofundi* (Gibson et al., 2005).

Many alkaliphiles are at the same time halophilic, and many useful enzymes applied in the detergent industry (washing powders), the textile industry, and other processes were obtained from bacteria growing in saline alkaline lakes. Halophilic enzymes (typical for halophilic archaea and bacteria) are characterized by an excess of acidic amino acids and subsequent negative surface charge. This feature allows effective competition for hydration water and enables function in solutions of low water activity, including organic solvent/water mixtures. The immediate advantages for enzyme technology are as follows: higher salt and heat tolerance, a catalytic environment which enables use of less polar educts, and potential reversal of hydrolytic reactions, all of which make them powerful candidates for industrial biocatalysts (Ma et al., 2010). The increase of salinity and nitrate/nitrite concentrations in soils and ground waters in the last few decades has focused much attention on the physiological and molecular mechanisms involved in salt-stress tolerance and nitrate metabolism by microorganisms. Physiological studies carried out with *Haloferax mediterranei* have revealed that it is resistant to very high nitrate (up to 2 M) and nitrite (up to 50 mM) concentrations (Bonete et al., 2008). Microorganisms are in general sensitive to low nitrate and nitrite concentrations. The inhibitory effect of these nitrogen compounds is due to the extreme toxicity of nitrite and nitric oxide produced upon nitrate reduction (Bonete et al., 2008).

3. Alkaliphilic Archaea

Alkaliphile are microorganisms that grow very well at pH values between 9 and 12 or grow only slowly at the near neutral pH value of 6.5 (Horikoshi, 1999). The best examples of naturally occurring alkaline environments are soda deserts and soda lakes. Extremely alkaline lakes, for example, Lake Magadi in Kenya and the Wadi Natrun in Egypt, are probably the most stable highly alkaline environments on Earth, with a consistent pH of 10.5 to 12.0 depending on the site (Horikoshi, 1999). Alkaliphilic haloarchaea are a specialized group of obligate extreme halophiles that require high salinity as well as high pH (8.5-11) and low Mg^{2+} for growth (Kamekura et al., 1997; Xu et al., 2001). Alkaliphilic haloarchaea are commonly isolated from soda lakes but they have also been isolated and detected from solar salterns and other hypersaline environments with acidic, neutral and alkaline pHs (Gareeb & Setati, 2009).

Alkaliphilic halophilic archaea are classified in the genera *Natronobacterium*, *Natronococcus* and *Natronomonas* (Kamekura et al., 1997), and some recent isolates are accommodated in *Halalkalicoccus* (Xue et al., 2005) and *Natronolimnobius* (Itoh et al., 2005). These five genera are so far composed exclusively of alkaliphilic strains. On the other hand, the genera *Haloarcula*, *Natronorubrum*, *Halobiforma*, *Natrialba*, *Haloterrigena*, *Halorubrum* and *Halostagnicola* consist of both neutrophilic and alkaliphilic species. *Halorubrum* and *Haloarcula* spp often form the majority of isolates is inhabited neutral brines (Grant et al., 2001). In a recent study isolates belonging to the genera *Natrialba*, *Natronococcus* and *Natronorubrum* were recovered from brine samples at evaporator ponds in Botswana (Gareeb & Setati, 2009).

Hypersaline soda lakes are mostly inhabited by alkaliphilic representatives of halophilic archaea that could be in numbers of 10^7 to 10^8 /ml in soda lake brines (Horikoshi, 1999). A novel haloalkaliphilic archaeon from Lake Magadi was isolated and characterized (McGenity & Grant, 1993). It was revealed that cells of this isolate contained large gas vacuoles in the stationary phase of growth, and colonies produced by these archaea were bright pink. Xu et al. (1999) isolated two haloalkaliphilic archaea from a soda lake in Tibet. The strains were gram negative, pleomorphic, flat, nonmotile, and strictly aerobic. Their growth required at least 12% (2 M) NaCl and occurred between pH 8.0 and 11 with an optimum at pH 9.0 to 9.5. DNA-DNA hybridization results suggested that the two strains belonged to different species of the same genus. Recently, A novel haloalkaliphilic archaeon from commercial rock salt imported to Japan from China was isolated and characterized (Nagaoka et al., 2011). The isolation of a haloalkaliphilic archaea, grown optimally at pH 10.0, from a saline–alkaline soil was reported (Wang et al., 2010).

The isolates displayed typical haloalkaliphilic growth characteristics with optimal growth at pH 9–10. Halophilic methanogens were isolated from various neutral saline areas and natural hypersaline environments (Boone et al., 2001). These strains showed the optimal growth at temperatures near 40°C and, in medium containing 0.5 to 2.5 M NaCl, at pH values near 7. Zhilina and Zavarzin (1994) described bacterial communities which inhabited in alkaline lakes, and in particular the diversity of anaerobic bacteria developing at pH 10 was exhibited. A new obligate alkaliphilic, methylotrophic methanogen was isolated from Lake Magadi (Kevbrin et al., 1997). Based on its phenotypic and genotypic properties, the isolate found to be belonged to *Methanohalophilus zhilinaeae*. It was an obligate alkaliphile and grew optimally within pH 9.2.

The alkaliphiles are unique microorganisms, with great potential for microbiology and biotechnological exploitation. The essential use of alkaliphilic enzymes is in the detergent industry, for example, an extracellular protease from *Natrialba magadii*, a haloalkaliphilic archaeon, is a solvent tolerant enzyme and suggests a potential application in aqueous-organic solvent biocatalysis (Diego et al., 2007). Recently it was reported that the gene encoding the protease Nep secreted by the *Natrialba magadii* was cloned and sequenced (DeCastro et al., 2008). The study was announced the molecular characterization of a halolysin-like protease from alkaliphilic haloarchaea and the description of a recombinant system that facilitated high-level secretion of a haloarchaeal protease. Alcohol dehydrogenase is a key enzyme in production and utilization of ethanol. The gene encoding for ADH of the haloalkaliphilic archaeon *Natronomonas pharaonis* was cloned and expressed in *Escherichia coli* (Cao et al., 2008). The enzyme was haloalkaliphilic and thermophilic, being most active at 5 M NaCl or 4 M KCl and 70°C, respectively. The optimal activity was

observed at pH 9.0 and significantly inhibited by Zn^{2+}. It was concluded that the physiological role of this enzyme is likely related to the oxidation of ethanol to acetaldehyde.

4. Acidophilic Archaea

Both natural and man-made acidic environments on the Earth are commonly found in the sites like pyrite ores, solfatara fields and marine volcanic vents; the microorganisms that inhabited these areas are called acidophiles and have a pH optimum for growth pH <3 (Baker-Austin & Dopson, 2007). Acidophiles are most widely distributed in the bacterial and archaeal domains and contribute to numerous biogeochemical cycles including the iron and sulfur cycles.

Acidophiles might have played some crucial function in the evolution because metabolic processes might have originated on the surface of sulfide minerals (Wachtershauser, 2006) and structuring of the genetic code could have occurred in an intracellular environment with acidic pH (Di Giulio, 2005). Acidophiles optimally grow in low pH and metal-rich environments which might be quite resemble to volcanic aqueous conditions during Archaean and early Proterozoic periods. Therefore, it was suggested that acidophiles could represent primordial form of life from which more complex life have evolved (Baker-Austin & Dopson, 2007). Acidophiles are mostly found in isolated and inaccessible environments like geothermal vents. These environments generally have an impassable physical barrier which reduces the growth of neutrophiles. Recent bioinformatic analysis of several thermoacidophile archaeal genomes have implied that the similarities between these organisms are greater than expected when compared with other more closely related organisms. Therefore, acidic environments could establish an old and genetically distinct niche of life in which ecological closeness disregards phylogenetic nearness (Futterer et al., 2004).

The ongoing exploration of the Earth has led to continued discoveries of life in environments that were previously considered uninhabitable. Thus, interest in the biodiversity and ecology of extreme environments and their inhabitants has grown over the past several years. In this regard, the study of extremely acidic environments is taking too much attention, because environmental acidity is often a consequence of microbial activity (Hallberg & Johnson, 2001). Highly acidic environments are relatively less common on Earth and are generally associated with mining activities. One of the main sources of acidity is the natural oxidation and dissolution of sulfidic minerals exposed to oxygen and water and this process can be greatly enhanced by microbial metabolism (Nordstrom & Alpers, 1999). Jhonson (1998) claimed that microorganisms that is involved in the generation of acidic metalliferous wastes cause widespread environmental pollution.

The extremely thermoacidophilic archaea are a group of interesting microorganisms in that they have to simultaneously cope with biologically extreme pHs and temperatures in their natural environments (Auernik et al., 2008). The current studies of the thermophilic and mesophilic acidophilic archaea have implied that there might be a stronger association between tetra-ether lipids and the tolerance to acid gradients than previously thought (Macalady & Banfield, 2003). Archaeal cell membrane including tetra-ether lipids instead of bacterial ester linkages is an example of highly impermeable cell membrane. These compounds were identified in *Thermoplasma acidophilum* (Shimada et al., 2002). Their

expanding biotechnological significance relates to their role in biomining of base and precious metals and their unique mechanisms of survival in hot acid, at both the cellular and biomolecular levels. Extreme thermoacidophiles are microorganisms that are characterized by having an optimal growth temperature ≥ 60 ^{o}C and an optimal pH of ≤4. A majority of the extremely thermoacidophilic species studied to date belongs to the archaeal orders of *Sulfolobales* and *Thermoplasmatales* (Auernik et al., 2008). *Acidianus infernus* which is the most thermophilic of the extreme thermoacidophiles grows at temperatures up to 95 ^{o}C and at pHs as low as 1.0. On the other hand, *Picrophilus* species, member of the *Thermoplasmatales* (euryarchaeon), are the most acidophilic organisms growing at pHs as low as 0 and at temperatures up to 65 ^{o}C (Huber & Stetter, 2006).

The application of recent molecular genetic systems and genome sequence data have given new clues in understanding of heavy metal tolerance, implementation of a genetic system and discovery of a new carbon fixation pathway. As a consequence, new insights into the molecular mechanisms that define extreme thermoacidophily have been gained. Extreme thermoacidophiles have evolved mechanisms for tolerating heavy metals which are physiologically toxic to most microorganisms (Salzano et al., 2007). These mechanisms cover to their capacity to recover from metal-induced damage (similar to oxidative stress) and to stop the accumulation of effective toxic metal concentration. In some cases, extreme thermoacidophiles could reduce or oxidize metals to less toxic forms by metabolic pathways. In other cases, metal chelation or complexation can perform the same job. Other mechanisms are based on the exporting toxic metal ions via P-type ATPases, instead of direct or indirect metal transformation (Ettema et al., 2006).

Acidophiles have some important biotechnological applications like metal recovery from ores, known as biomining (Rawling, 2002). Biomining is becoming increasingly important in mining because of its lower and containable pollutant outputs comparing to thermal processes. Efficacy in biomining environments demands tolerance to high levels of toxic heavy metals and the ability to incorporate inorganic carbon, as organic carbon can be scarce in this environment (Auernik et al., 2008). The future success of the biomining industry will depend upon the cellular biocatalysts with favorable features. The knowledge coming from genomic research of extreme thermoacidophiles will open new applications in this area.

5. Methanogens

Methane (CH_4) is an important greenhouse gas and its atmospheric abundance has been increasing by about 0.5% per year. Methanogenesis is a common process in many anaerobic environments such as digesters, rumen, rice fields, oil wells, landfills, and a range of extreme habitats (Garcia et al., 2000). Microorganisms are considered to be responsible for about the 50% of total methane production in the world. Methanogens, CH_4-producing microorganisms, are strict anaerobes in the *Euryarchaeota* and can produce CH_4 from a limited number of substrates: CO_2 and H_2, formate, methanol, methylamines or acetate. As terminal oxidizers in complex microbial communities, they are vital to the anaerobic microbial degradation of organic compounds in natural environments and probably also in defined ecological niches of the human body (Cavicchioli et al., 2003). Since methanogens coexist and closely interact with anaerobic bacteria at certain sites (e.g., human colon or dental plaque) they could be implicated in mixed anaeorobic infections. In fact,

methanogens have recently been linked to periodontal disease (Lepp et al., 2004), a polymicrobial infection that affects the gums and supporting structures of the teeth and is characterized by periodontal pockets. Methanogens are phylogenetically diverse organisms although they rely on few metabolic substrates (Yavitt et al., 2011).

Methanogens are located in the domain *Archaea* and the phylum *Euryarchaeota*. Unlike *Bacteria*, some methanogens have pseudomurein in their cell wall instead of peptidoglycan (Balch et al., 1979). The glycan strand of pseudomurein is composed of alternating β-(1→3)-linked N-acetyl-D-glucosamine and β-(1→3)-linked N-acetyl-L-talosaminuronic acid (Kandler & Konig, 1998). The most closely related bacterial homologs to pseudomurein have been found in anaerobic Gram-positive or *δ*-proteobacterial lineages. It is reported that these organisms share the same ecological niche, and could also be the donors of peptide ligase homologs (MurC, MurE and MurF) which are probably responsible for pseudomurein biosynthesis in the *Methanobacteriales* (Graham & Huse, 2008). While pseudomurein is a common cell wall component in *Methanobrevibacter* and *Methanobacterium*, heteropolysaccharide is in *Methanosarcina,* and protein is in *Methanococcus* and *Methanocalculus*. Methanogens are also characterized by including coenzyme F420, which is a cofactor necessary for certain enzyme activities such as hydrogenase and formate dehydrogenase. Another coenzyme typical to methanogens is coenzyme M, which is either produced by the methanogens, such as *Methanobacterium*, or is obtained from an external source, which is the case for *Methanobrevibacter ruminantium* (Ashby et al., 2001).

Methanogens were the first organisms to be identified as archaea and classified as a separate domain. Six orders of methanogens exist: *Methanobacteriales*, *Methanopyrales*, *Methanococcales*, *Methanomicrobiales*, *Methanosarcinales* and *Methanocellales*. All of these orders contain a wide diversity of taxa that display great variance in their morphological and physiological characteristics. However, they all retain in common an anaerobic lifestyle and the ability to produce methane metabolically (Bapteste et al., 2005). Complete genome sequences for representatives of all of these orders are available. Initial analyses of the *Methanocaldococcus* - formerly *Methanococcus jannaschii* genome have revealed that the archaea have many metabolic characteristics in common with bacteria, but that the genes used for information processing are more similar to equivalent systems in eukaryotes (Walters & Chong, 2010).

Following the suggestion that the *Archaea* are a distinct taxonomic group, it was considered that the domain would be divided along phenotypic lines and that the methanogenic archaea would be monophyletic (Bapteste et al., 2005). The 16S rRNA gene comparisons, however, indicated that this was not the case; the *Methanomicrobiales* were more closely related to extremely halophilic archaea (*Halobacteriales*) than to other methanogens (Woese et al 1990). The isolation of *Methanopyrus kandleri* and the sequencing of its 16S rRNA gene sequences showed that it was unrelated to any other known methanogens and originated at the base of the euryarchaeal branch of the tree, implied that the ancestor of euryarchaeal species might have been a methanogen (Burggraf et al., 1991). Recent studies based on transcriptional and translational proteins and 16S rRNA sequences have approved the absence of monophyly of methanogens and strongly suggested a close phylogenetic relationship between *Methanopyrus*, *Methanococcus* and *Methanothermobacter* (Brochier et al., 2004).

As most of archaea have been found living at the extreme sites on this planet, they have often been proposed to resemble the life outside the earth if exists (Jarell et al., 2011). The

expectations of Earth-like organisms that could exist on other planets has varied, with methanogens often mentioned because of their adaptation to anaerobic niches with little or no organic carbon (Moissl-Eichinger, 2011), and especially with respect to the possible biogenic formation of the methane on Mars. Methanogenesis is a process occurring in many anaerobic environments. For instance methanogenesis in cold marine sediments has a global significance by leading to methane hydrate deposits, cold seeps, physical instability of sediment, and atmospheric methane emissions. The analysis of cultivation-independent archaeal community revealed that uncultivated microbes of the kingdoms *Euryarchaeota* and *Crenarchaeota* are present and that methanogens comprised a small proportion of the archaeal community. Methanogens were cultivated from depths of 0 to 60 cm in the sediments, and several strains related to the orders *Methanomicrobiales* and *Methanosarcinales* were isolated (Kendall et al., 2007). Microbial diversity was examined in the cold marine sediments (Orphan et al., 2002), however, the function that these microbes carry out during geochemical processes is still not clear. In addition to methanogens, uncultivated lineages of other *Archaea* have been also identified in marine sediments (Vetriani et al., 1999).

Unlike that of bacteria, the diversity of gut methanogenic archaea seems to be well understood and limited to two species belonging to *Methanobacteriales*, one of the five methanogenic orders defined to date: *Methanobrevibacter smithii* and occasionally *Methanosphaera stadtmanae* (Miller & Volin, 1982). In a current study the diversity of methanogenic Archaea from the gut of humans was analyzed by targeting mcrA, a molecular metabolic marker of methanogenesis (Mihajlovski et al., 2008). They reported the presence of *Methanobacteriales*, *Methanobrevibacter smithii*, *Methanosphaera stadtmanae* and a distant phylotype that did not cluster with any of the methanogenic orders. Their results were also supported by 16S archaeal sequences retrieved from the same volunteer, strongly suggests there may be a sixth order and hence potential underestimation of the role of methanogens in gut physiology. There is a link between the physiology of cultured methanogenic Archaea and their phylogenetic closeness based on 16S rRNA sequences (Zinder, 1993). For example, while most species of the *Methanobacteriaceae* and *Methanomicrobiaceae* prefer H_2 and CO_2 (or formate) as substrates for methanogenesis, *Methanosaeta*, a genus within the *Methanosarcinaceae*, is known to generate energy only from acetate fermentation. Most of the other *Methanosarcinaceae* preferentially use methanol and related methyl-substrates for the generation of CH_4 (Kleikemper et al., 2005).

It has been considered that methanogenesis may function in the mineralization of petroleum hydrocarbons in contaminated aquifers (Chapelle et al., 2002). However, petroleum hydrocarbons can not be degraded directly by methanogenic microorganisms (Zengler et al., 1999). Methanogens metabolizing H_2 and CO_2 involve indirectly into PHC degradation by maintaining H_2 concentrations low by that way fermentation of PHC becomes exergonic and fermenting microrganisms can grow (Garcia et al., 2000). On the other hand, methanogens metabolizing acetate or methanol can degrade PHC directly by cleaving end products of fermentation. However, the role of different metabolic groups of methanogens with respect to overall methanogenic activity in PHC-contaminated aquifers is reported to be unclear (Kleikemper et al., 2005). Aceticlastic methanogenesis was assumed to be the final step of hydrocarbon degradation in a PHC-contaminated aquifer, but this was not confirmed with activity measurements (Dojka et al., 1998). The research for alternative forms of energy, including recovery of methane via anaerobic digestion of wastes, has becoming very popular since successive petroleum crisis in 1970s.

6. Thermophilic Archaea

In the domain Archaea, hyperthermophilic and extremely thermophilic archaea are determined extensively over the phyla *Crenarchaeota* and *Euryarchaeota*. Hyperthermophiles, growing optimally at ≥ 80 °C, have been acknowledged since 1981 (Zillig et al., 1981). They represent the upper temperature limit of life and are found in environments characterized with high temperature. In their basically anaerobic environments, they generally obtain energy by inorganic redox reactions. Stetter (2006) claimed that in his lab so far about 50 new species of hyperthermophiles have been isolated and characterized, among them representatives of the novel bacterial genera *Thermotoga*, *Thermosipho*, *Aquifex*, *Thermocrinis* and archaeal genera *Acidianus, Metallosphaera, Stygiolobus, Thermoproteus, Pyrobaculum, Thermofilum, Desulfurococcus, Staphylothermus, Thermosphaera, Ignicoccus, Thermodiscus, Pyrodictium, Pyrolobus, Thermococcus, Pyrococcus, Archaeoglobus, Ferroglobus, Methanothermus, Methanopyrus* and *Nanoarchaeum*. The earliest archaeal phylogenetic lineage is represented by the extremely tiny members of the novel kingdom of *Nanoarchaeota* (Stetter, 2006). Hyperthermophiles occupy all the short deep branches closest to the root within the universal phylogenetic tree. In rRNA-based phylogenetic tree constructed by Woese et al. (1990), all extremely short and deeply branching-off lineages within the archaea and bacteria are exclusively represented by hyperthermophiles (Fig. 1), indicating a slow rate of evolution (Stetter, 2006).

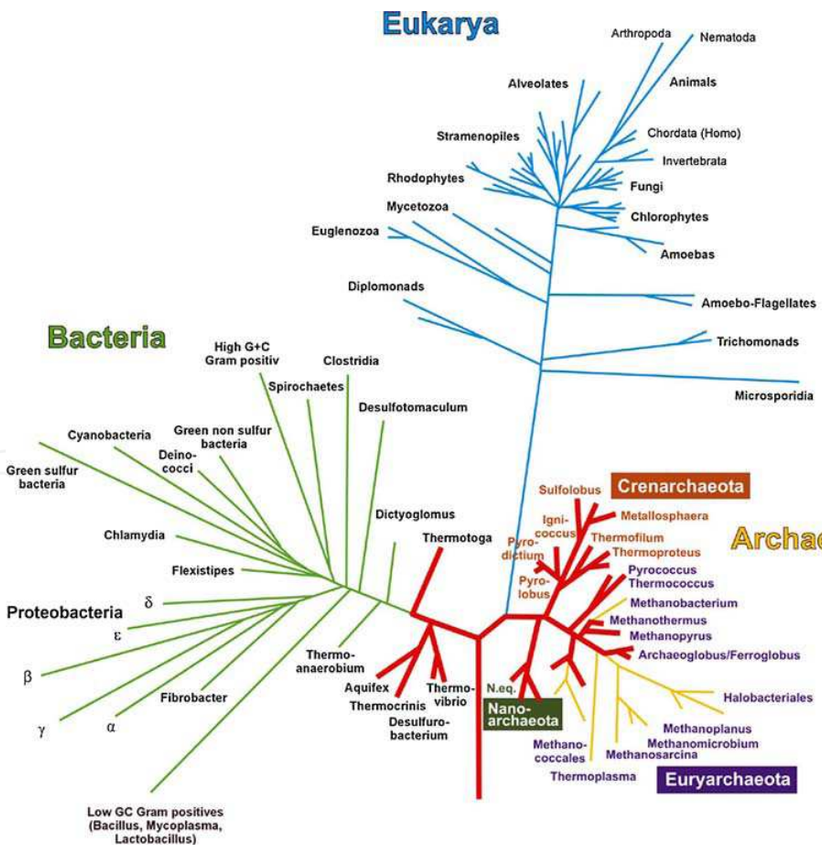

Fig. 1. Universal phylogenetic tree constructed based on rRNA sequence comparison. Hyperthermophiles represented with thick red lineages (Stetter, 2006).

Until now, all *Crenarchaeota* species that have been isolated are either hyperthermophilic or extremely thermophilic, although the existence of mesophilic and psychrophilic *Crenarchaeota* is also suggested by culture-independent molecular phylogenetic analyses. The cultured *Crenarchaeota* are composed of five orders: *Acidilobales, Fervidicoccales*, *Desulfurococcales*, *Sulfolobales* and *Thermoproteales*, which are well-supported by 16S rDNA sequence data and by phenotypic properties, such as cell morphology and lipid composition (Burggraf et al., 1997; Reysenbach, 2001; Chaban et al., 2006; Prokofeva et al., 2009). Some of the crenarchaeotas are sequenced from low-temperature environments (Dawson et al., 2001). So far only one member of the groups, *Crenarchaeum symbiosum*, from non-thermophilic environments has been cultivated in axenic culture (Preston et al., 1996). The information about non-thermophilic *Crenarchaeota* is, therefore, solely based on sequence data collected from various low temperature environments like soil, freshwater, deep drillings, and seawater.

The analyses about the energy sources of hyperthermophiles have revealed that most species are chemolithoautotroph (Amend & Shock, 2001; Stetter, 2006). Mode of Respiration is anaerobic -nitrate, sulphate, sulphur and carbon dioxide respiration- and aerobic, CO_2 is the solely carbon source required to synthesize organic cell material. While molecular hydrogen generally serves as main electron donor, sulphide, sulphur, and ferrous iron are other electron donors. Oxygen also may serve as an electron acceptor in some hyperthermophiles which are usually microaerophilic. Anaerobic respiration forms are the nitrate-, sulphate-, sulphur- and carbon dioxide respirations. While chemolithoautotrophic hyperthermophiles synthesize organic matter, some obligate heterotrophic hyperthermophiles depend on organic material as energy- and carbon-sources. In addition, a few chemolithoautotrophic hyperthermophiles are opportunistic heterotrophs. They obtain energy either by aerobic or anaerobic respiration or by fermentation (Stetter, 2006).

Recently a novel phylum of Archaea, called *Nanoarchaeota*, was discovered. The phylum is currently represented by a single species named as *Nanoarchaeum equitans*. The species is a nano-sized hyperthermophilic symbiont that grows attached to the surface of an *Ignicoccus* species (Huber et al., 2002). It has a cell diameter of only 400 nm and grows under strictly anaerobic conditions at temperatures between 75 °C and 98 °C. The *N. equitans* has a genome of only 490.885 bp, it is the smallest genome of any Archaea, and the most compact, with 95% of the DNA predicted to encode proteins or stable RNAs. Its DNA encodes the complete machinery for information processing and repair, but lacks genes for lipid, cofactor, amino acid, and nucleotide biosynthesis. The inadequate biosynthetic and catabolic capacity of *N. equitans* considers its symbiotic relationship to its *Ignicoccus* host as a parasitic relationship, and this makes *N. equitans* the only known archaeal parasite (Waters et al., 2003).

7. Biotechnological importance of Archaea

Industries such as food processing, cleaning, biosynthetic processes and environmental bioremediation need efficient biocatalysts which can operate in harsh environments. The sources for these biocatalysts have been animals, plants, fungi and mostly bacteria. Recently, the extremophilic bacteria and archaea have become more popular since the enzymes of these organisms are able to remain catalytically active under extremes of temperature, salinity, pH and pressure (Synowiecki et al., 2006; Ozcan et al., 2009). In biotechnology for

an efficient application, one has to determine the most suitable enzymes and best reaction conditions. Nowadays there are two main strategies for obtaining enzymes with desired properties, namely the genetic engineering of currently known enzymes and the search for new activities in previously uncharacterized microorganisms. Within the second approach, the search for enzymes in extremophiles (called extremozymes) seems to be particularly promising since the enzymes of these organisms have particular adaptations to increase their stability in adverse environments, which can potentially also increase their stability in the harsh environments in which they are to be applied in biotechnology (Oren, 2002).

The domain archaea is particularly under extensive research for their potential biotechnological applications. The domain includes the extreme halophiles, the hyperthermophiles, the thermoacidophilic archaea and the psychrophiles that live in the cold waters of the Antarctic. But the most important value of these organisms (along with some of the bacteria that also tolerate extreme environments) is that their enzyme systems work at harsh conditions. For example, many of the restriction enzymes used in gene splicing and cloning are products of extremophiles. In 1993 a report from US National Academy of Sciences noted that world enzyme sales equaled to US$1 billion and it is a market that has been expected to grow about 10% per year. It is expected that enzymes from extremophiles will constitute an important part of this market.

Recently genes encoding several enzymes from extremophiles have been cloned in mesophilic hosts, with the objective of overproducing the enzyme and altering its properties to suit commercial applications (Alqueres et al., 2007). It has been revealed that enzymes derived from extremophilic archaea are in many cases superior to bacterial homologs. They have higher stability towards heat, pressure, detergents and solvents (Egorova & Antranikian, 2005). The archaeal hyperthermophiles, known as heat-stable prokaryotes, have exceptionally high growth temperature limits and unique ether-linked lipids as well as eukaryotic transcription and translation factors. It has been proposed that heat shock proteins which are inducible by supraoptimal temperatures (Kagawa et al., 1995) and reverse gyrase which installs positive supercoils in DNA (Guipaud et al., 1997) could have been involved in unusual adaptive mechanisms of the hyperthermophiles.

DNA polymerase I from the bacterium *Thermus aquaticus*, Taq polymerase, is the first thermostable DNA polymerases in biotechnology and has an extensive use in PCR. For PCR applications, the archaeal DNA polymerases have been found to have some superior features to bacterial ones. For instance, Pwo from Pyrococcus woesei, Pfu from P. furiosus, Deep Vent polymerase from the *Pyrococcus* strain GB-D and Vent polymerase from *Thermococcus litoralis*, reported to have an error rate that is much lower than that of Taq polymerase.

The starch-processing industry needs thermostable enzymes in order to convert starch into more valuable products such as dextrins, glucose, fructose and trehalose, (Egorova & Antranikian, 2005). It is well established that in all starch-converting processes, high temperatures are required to liquefy starch and to make it accessible to enzymatic hydrolysis. The synergetic action of thermostable amylases, pullulanases and α-glucosidases include the advantage of lowering the cost of making sugar syrup, production of new starch-based materials that have gelatin-like characteristics and defined linear dextrins that can be used as fat substitutes, texturizers, aroma stabilizers and prebiotics. Recently a variety of starch-degrading enzymes from extremophilic archaea has been published. They

determined the optimal temperatures for the activity of the archaeal amylases in a range between 80 °C and 100 °C. Especially, the high thermostability of the extracellular α-amylase from *Pyrococcus* sp. (retain its activity even at 130°C) makes these enzymes ideal candidates for industrial application (Egorova & Antranikian, 2005).

Lipases (carboxyl ester hydrolases) are ubiquitous in nature, produced by animals, plants, and fungi, as well as bacteria. Recently halophilic archaeal organisms are taking more attention due to their lipolytic enzymes. For instance, *Haloarcula marismortui*, a halophilic archaeon whose genome was sequenced contains genes encoding for putative esterase and lipase. These genomic predictions have been verified recently (Camacho et al. 2009). They reported that *Haloarcula marismortui* displays esterase and lipase activity intracellularly and extracellularly. While lipase was accumulated mainly extracellular, esterase was generally accommodated intracellular (Camacho et al., 2009). The esterase and lipase genes of *Pyrococcus furiosus*, a hyperthermophilic archaeon, have been cloned in *E. coli* and functional properties have been determined. The archaeal enzyme reported as the most thermostable and thermoactive esterase known to date (Ikeda and Clark 1998). In our laboratory, the halophilic archaeal isolates, grown best in a range of 2-5 M NaCl, produced high lipolytic activity in the range of 3-4.5 M NaCl (Ozcan et al., 2009). It was found that the lipolytic activity dropped at 5 M NaCl. Therefore these enzymes can be classified as not only salt dependent but many can be also thermostable (Oren, 2002; Ozcan et al., 2009).

Heat-stable proteases have some utilities in biotechnology, especially in the detergent industry. It has been revealed that most proteases from extremophilic Archaea belong to the serine type and are stable at high temperatures, even in the presence of high concentrations of detergents and denaturing agents (Antranikian et al., 2005). Proteases are also applied for peptide synthesis using their reverse reaction, mainly because of their compatibility with organic solvents (Egorova & Antranikian, 2005). Recently a protease from *Thermococcus kodakarensis* has been characterized (Foophow et al., 2010). It has been shown that a β-jelly roll domain is not directly involved in proteolytic activity, however, takes role in the extreme thermostability of the enzyme.

One of the important strategies in searching the alternative energy sources could be the conversion of biomass to fuel products (biofuels). Both chemical and biological processes are being explored for the production of bioethanol, biodiesel, biobutanol, biomethane and biohydrogen. For the production of biohydrogen some heterotrophic hyperthermophiles particularly have been found useful because of their abilities to produce molecular hydrogen (Atomi et al., 2011). It is covalently linked to Lys216 in the chromophore by Schiff base action.

Habitats with harsh environmental conditions are often populated by archaea that are specialized to live in water near salt saturation also specialized to utilize solar energy. Light of distinct wavelengths is used to fuel primary transport of both protons and chloride ions as well as for phototaxis. The halophilic archaeon makes use of light for both energy and sensory transduction by exploiting a family of light-sensitive proteins called rhodopsin. Bacteriorhodopsin is a 25-kDa integral membrane protein that carries a retinal group (cromophore) and it is covalently linked to lysine-216 by Schiff-base action. Its function was discovered in the early 1970s during studies of the purple membrane, patches of membrane that contain only bacteriorhodopsin and lipids, found within the cell membrane of *Halobacterium salinarum* (Oren, 2008). The excellent thermodynamic and photochemical

stability of bacteriorhodopsin has led to many uses in technical applications like holography, spatial light modulators, artificial retina, neural network optical computing, and volumetric and associative optical memories (Alqueres et al., 2007).

Archaeosomes, a kind of liposomes, are produced from natural lipids found in *Archaea* or from synthetically synthesized compounds that share the unique structural features of archaeal lipids. The isoprenoid glycerolipid membrane of archaeosomes can develop into a bilayer, a monolayer, or a combination of mono- and bilayers made from bipolar and monopolar archaeal lipids (Jacquemet et al., 2009). The archaeal lipids formulations have shown to exhibited relatively higher physico-chemical stabilities to oxidative stress, high temperature, alkaline pH, action of phospholipases, bile salts and serum media (Brard et al., 2007). These properties contribute to their efficacy as self-adjuvant vaccine delivery vesicles. Additionally, archaesomes were found to be safe and not toxic in mice both in-vitro and -vivo studies (Omri et al., 2003). Thus, it has been claimed that the biocompatibility and the superior stability properties of archaeosomes in several conditions give advantages over conventional liposomes in the manufacture and the use in biotechnology including vaccine and drug delivery (Jacquemet et al., 2009).

8. Conclusion

The majority of the living beings thrive in environments having physically and geochemically temperate conditions. The extreme environments found on the planet are generally inhabited by microorganisms, which belong to the Archaeal and Bacterial domains of life. Archaea, members of the third domain of life, are prokaryotes that harbour many unique genotypic and phenotypic properties. Two archaeal phyla are presently recognized; the *Euryarchaeota* and the *Crenarchaeota* (Woese et al., 1990).

Archaea exhibit a wide diversity of phenotypes. The first phenotypes to be recognized were the methanogens which are strict anaerobes and methane producers. Many archaea are extremophiles which are capable of growth at high tempereture, salinity and extremes of pH. In common usage, cultivated *Archaea* were, without exception, considered to be extremophiles. Extreme environments comprise sites of extreme temperature, pH, pressure and salinity. *Archaea* exist in a broad range of habitats, and as a major part of global ecosystems, may contribute up to 20% of earth's biomass (Delong and Pace, 2001).

Thermophilic archaea inhabit environments like hot springs, ocean vents, and geysers which are inhospitable to many other organisms. These habitats not only have extremely high temperatures but have high concentrations of dissolved minerals and low concentrations of oxygen (Egorova and Antranikian, 2005). In particular, enzymes from thermophilic and hyperthermophilic Archaea have industrial relevance. Enzymes of thermophilic archaea are superior to the traditional catalysts because they can perform industrial processes even under harsh conditions (Egorova and Antranikian, 2005).

The extreme halophilic archaea require at least 1.5 M NaCl. Most strains grow best at 3.5–4.5 M NaCl. Members of the halophilic archaea are dominant microorganisms in hypersaline environments worldwide including salt lakes, crystallizer ponds of solar salterns, salt mines, as well as hypersaline soda lakes (Oren, 2002). Halophilic archaea have a number of useful applications in biotechnological processes and potential new applications are being investigated. For instance, they produce bacteriorhodopsin (used in information processing

and ATP generation), novel extracellular polysaccharides, exoenzymes (amylase, cellulase, xylanase, lipase and protease) and poly-hydroxyalkanoate (used in biodegradable plastic production), and a protein from *Halobacterium salinarum* has significance in cancer research. Some members are extremely alkaliphilic as well as being halophilic. They grow optimally in alkaline environment with pH values above 9 such as soda lakes and carbonate-rich soils but cannot grow or grow only slowly at the near-neutral pH value (6.5). On the other hand alkalithermophilic members of the Archaea were isolated besides halophilic. These oorganisms have economic value because alkaliphilic enzymes have been used in different industries (Horikoshi, 1999).

Acidophilic archaea have a pH optimum for growth of less than pH 3. They contribute to numerous biogeochemical cycles including the iron and sulfur cycles. Acidophiles have different biotechnological applications. Firstly metal extraction from ores and this sustainable biotechnological process is becoming increasingly important because of its reduced and containable pollutant outputs. Acidophiles could also be a source of gene products; for example, acid-stable enzymes with applications as lubricants and catalysts (Baker-Austin and Dopson, 2007).

Metabolism of methanogens is unique in that energy is obtained by the production of methane (natural gas). Biological methanogenesis is applied to the anaerobic treatment of sewage sludge, and agricultural, municipal and industrial wastes, where the maintenance of a desired methanogenic flora is achieved by inoculation (Schiraldi et al., 2002).

Many archaea colonize extreme environments. Because extremophilic microorganisms have unusual properties, they are a potentially valuable resource in the development of novel biotechnological processes. Especially, based on the unique stability of archaeal enzymes at high temperature, salt and extremes of pH, they are expected to be a very powerful tool in industrial biotransformation processes that run at harsh conditions.

The growing demand for more effective biocatalysts has been satisfied either by improving the properties of existing proteins or by producing new enzymes. The majority of the industrial enzymes known to date have been extracted mostly from bacteria and fungi. Until now, only a few archaeal enzymes have been found to be useful in industrial applications. Based on the unique stability of archaeal enzymes at high temperature, salt and extremes of pH, they are expected to be a very powerful tool in industrial biotransformation processes that run at harsh conditions. Owing to the unique features of *Archaea*, their potential applications in biotechnology are far reaching, ranging from bioremediation potential of nitrite and nitrate from groundwater, oil pollutions, toxic compounds, metal polluted sites and biomining and nitrate removing from brines (Schiraldi et al., 2002).

9. References

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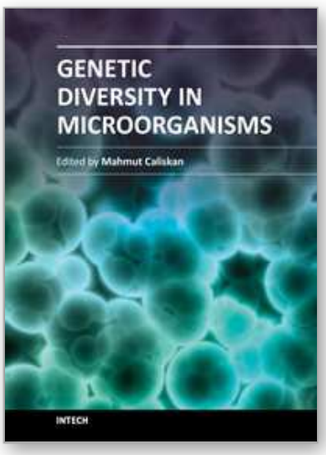

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Genetic Diversity in Microorganisms presents chapters revealing the magnitude of genetic diversity of microorganisms living in different environmental conditions. The complexity and diversity of microbial populations is by far the highest among all living organisms. The diversity of microbial communities and their ecologic roles are being explored in soil, water, on plants and in animals, and in extreme environments such as the arctic deep-sea vents or high saline lakes. The increasing availability of PCR-based molecular markers allows the detailed analyses and evaluation of genetic diversity in microorganisms. The purpose of the book is to provide a glimpse into the dynamic process of genetic diversity of microorganisms by presenting the thoughts of scientists who are engaged in the generation of new ideas and techniques employed for the assessment of genetic diversity, often from very different perspectives. The book should prove useful to students, researchers, and experts in the area of microbial phylogeny, genetic diversity, and molecular biology.

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