Chapter 16
Archaea: Ecology, Application, and Conservation

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Abstract Archaea are the inhabitants of extreme environments on the earth. They commonly live at extreme acidity, temperature, and alkalinity or in hypersaline water, hot springs, hydrothermal vents, and glaciers and at extreme pressure and radiation. Some of the members live in deep oceans at extreme pressure and temperature above 100 °C. With the advancement of archaeal taxonomy, diversity, and identification of new strains, their functional role has increased in industrial and biotechnological applications in recent years. The extremophilic archaea are well-known sources of extracellular enzymes and biocatalyst and accelerate fermentation process. Some novel antimicrobial compounds and biomolecules have been discovered in certain archaea. Many of archaeal strains have applications in eco-friendly wastewater treatment plants, biodegradation of marshy lands contaminated with organic solvents, and hydrocarbons. In mineralization process, ammonia-oxidizing archaea (AOA) has key role in nitrogen cycle. The long-term preservation of extremely halophilic and thermoacidophilic archaea has been reported successful by L-drying method but it is labile to freeze and freeze-drying. Viability of thermoacidophilic archaea like *Thermoplasma* sustained at 5 °C for more than 15 years. The halophilic archaea may be preserved in the Petri dishes or in the refrigerator at 4 °C for quite longer periods with proper sealing and in deep freezing at −80 °C with specific media at proper salt concentration in 20% supplemented glycerol. In the case of hyperthermophilic archaea like *Pyrococcus furiosus*, the glass capillary tube kept over liquid nitrogen with dimethyl sulfoxide is preferred. The lyophilization method of preservation generally results in loss of viability in most of archaea cultures. Likewise, in situ methods of conservation of archaea in their natural habitats become noteworthy since most of archaea are extremophiles in those particular habitats with unique characteristics and specific traits with several applications. Hence, preservation of archaea requires specific preservation techniques for certain groups, and therefore, it is important to be focused on their maintenance, preservation, and conservation. Hence, it is very important for the

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development of reliable, simple, and durable preservation technique for particular groups of archaea for long-term preservation with stable viability for over the years.

16.1 Introduction

The Archaea as a major domain of life was not considered for a long time. However, in the late 1970s, a team of Dr. Carl Woese at the University of Illinois was studying the relationships among the prokaryotes with the help of DNA sequences that suggested a new domain of organisms known as the Archaea. These researchers studied bacteria (prokaryotes) that are known to live at higher temperatures andproduce methane were clustered together as a separate group and placed as entirely in a new group different from those of bacteria and the eukaryotes. As there was wide genetic difference among the other domains, Dr. Carl Woese proposed that life form be divided into three domains as Eukaryota, Eubacteria, and Archaebacteria. Further, he suggested that the term Archaebacteria was a misnomer and should be restricted only to Archaea, as the organisms under this group have wide difference from others. The taxonomic classification of these three initial groups was based on basesequence studies of 16S and 18S ribosomal RNA (rRNA) molecules (Woese and Fox 1977). The word Archaea comes from the Ancient Greek thus meaning "ancient things" (http://www.merriam-webster.com/dictionary/archaea). It is considered that the Archaea originated from the common ancestor at the time of evolution and are therefore regarded as the most primitive group of organisms in the life form. The group of methanogens was in separate domain, and domain Archaea was placed in extremophiles found only in extreme habitats, i.e., hot springs, cold deserts, extreme pH, salt ponds, and hypersaline lakes. During the early twenty-first century, researchers and the microbiologists accepted that the Archaea are a large, new, and diverse group of organisms, widely distributed in nature, and are also common in non-extreme habitats, such as soils and oceans (DeLong 1998). It is seen that most of the Archaea under this domain group are highly adapted to extreme conditions and the group can be easily divided into hyperthermophiles, halophiles, and methanogens. Despite the morphological studies, the Archaea are biochemically more closely related to the Eukarya than to the Eubacteria (Bullock 2000).

16.2 Evolution of the Archaea

The Archaea are prokaryotes like bacteria and are members of the third domain of life which depicting many unique genotypic as well as phenotypic properties, testifying for their peculiar evolutionary status. It is a general perception that the archaeal ancestor was probably a hyperthermophilic anaerobe under evolutionary process (Forterre et al. 2000). Therefore, the evolution of organisms and Archaea as

distinct domain is an important field in the study of evolutionary biology. Thus, new domain Archaea has a vast range of phenotypic and genotypic characters; it would be an interesting field to study the historical background of the archaea domain, so as to understand the ancestral origin and evolution of these characters. With the extensive study of data from comparative genomics, we can now sum up with more traditional or conventional phylogenetic and taxonomic approaches. It is known that more than 12 genomes of Archaea have been completely sequenced and are now available in public databases (http://www.ncbi.nlm.nih.gov/PMGifs/Genomes/). Similarly, detailed description of identified archaeal species recently has been published in new Volume I of Bergey's Manual edition (Boone and Castenholz 2001).

Furthermore, archaeal rRNA probes have been developed which are widely been used by molecular biologist and ecologists to study and investigate the worldwide distribution of the organisms of this domain, as well as its phylogenetic relatedness (Massana et al. 2000; Lopez-Garcia et al. 2001). The phylogenetic relatedness of Archaea at molecular level is well-advanced, well-documented, and well-studied through comparative genomics (Olsen and Woese 1997; Forterre 1997; Fitz-Gibbon and House 1999; Forterre and Philippe 1999; Snel et al. 1999; Tekaia et al. 1999; Makarova et al. 1999; Wolf et al. 2001). In comparative genomics and evolutionary studies, among the archaeal genomes sequenced until now, it is seen that most encoded proteins match and give first hits with other archaeal proteins when their homologues are searched in public databases. This is very much true for informational proteins (those involved in DNA replication, transcription, and translation) which are usually present in most archaeal genomes and are very close between one archaeon and another compared to those between one archaeon and any other prokaryote or eukaryote organism.

These archaeal distinctive informational proteins are usually showing more similarity to those of eukaryote than to those of prokaryote (bacteria). It is generally believed that protein information comprise the "core" of any organism's genome, since they have less probability of lateral gene transfer (LGT) and therefore are considered as more representative of the ancestral or evolutionary closeness of the organisms (Jain et al. 1999). In contrast to this, there is a well-recorded LGT between archaea and bacteria in aminoacyl-tRNA synthetases where few LGTs of informational proteins have been identified between the two prokaryotic domains (Wolf et al. 1999; Woese et al. 2000).

16.3 Taxonomy of the Archaea

On the basis of rRNA analysis, there are two major groups within Archaea: the kingdom Crenarchaeota and Euryarchaeota. The third kingdom Korarchaeota branches off close to the root (Grant and Larsen 1989; Dawson et al. 2006). A fourth kingdom Nanoarchaeota has been recently discovered in 2002 (Huber et al.

2002). More recently the new kingdom Thaumarchaeota has been proposed in 2008 and 2011 (Tourna et al. 2011; Brochier-Armanet et al. 2008).

A. Crenarchaeota

The kingdom Crenarchaeota contains organisms that live in extreme temperatures like very hot and very cold environments. Most of the members under culturable crenarchaeotes are hyperthermophiles. The members of hyperthermophilic archaea have been isolated from geothermal soils, water, or wastes containing elemental sulfur, sulfides, heavy metals, and solvents. On contrary to the hyperthermophiles, crenarchaeotes under extreme cold have been identified by the analysis of community sampling of ribosomal RNA genes from many nonthermal environments and/or habitats. The developments of fluorescent phylogenetic probes have enabled to find crenarchaeotes in marine waters worldwide. However, these marine crenarchaeotes thrive even in frigid waters, such as those of the Arctic and Antarctic. These organisms are planktonic in nature and occur in significant numbers (~10^4/ml) in waters that are nutritionally very poor and even under very cold condition (Madigan et al. 2009). Despite the members are found in sulfur-rich hot springs, the environmental rRNA indicated that they are most abundant in marine habitats (Madigan and Martinko 2005).

B. Euryarchaeota

The kingdom Euryarchaeota consists of a wide range of ecological archaeal diversity which includes variety of characteristics group like hyperthermophiles, methanogens, halophiles, and thermophilic methanogens. Also, a large group of uncultured marine Euryarchaeotes is included in this kingdom. The members of this group are mainly separated from other archaea on the basis of rRNA gene sequences. They may be either gram positive or gram negative and differentiate on the basis of presence of pseudomurein in the cell wall. The diverse archaeal groups like methanogens are obligate anaerobes. The members under these archaeal groups are known to thrive under anaerobic environments and habitats including seawater and freshwater bodies, deep soils, intestinal tracts of animals, industrial processing plants, and sewage treatment facilities. Extremely halophilic archaea or haloarchaea are among the diverse group of prokaryotes that inhabit under hypersaline niches or environments such as crystallizer ponds, saltern pans, solar salt evaporation ponds and natural salt lakes, or artificial saline habitats such as the surfaces of heavily salted foods like certain fish, marine food products, and meats. Such habitats are often called hypersaline. Extreme halophilic archaea are mostly aerobic. These organisms require high salt concentrations for growth and development, however, in some cases near saturation point (Madigan et al. 2009). Currently widely accepted taxonomy is based on *List of Prokaryotic names with Standing in Nomenclature (LPSN)*, *NCBI* database, and 16S rRNA-based LTP release 121 (full tree) by "The All Species LTP."

C. Korarchaeota

The 16S rRNA gene analysis revealed that phylogenetic lineage is not closely related to common archaeal groups, i.e., Crenarchaeota and Euryarchaeota, therefore, suggesting deep branching lineage (Elkins et al. 2008). The members of

Korarchaeota are only found in hot springs and hydrothermal vents, but low in numbers. They are found in habitats like iron- and sulfur-rich Yellowstone hot spring in Obsidian Pool, USA, and hot springs of Kamchatka, Russia, etc.

The Korarchaeota Kingdom of hyperthermophilic archaea is located very close to the archaeal root. It is a part of archaeal TACK superphylum comprising major archaeal groups (Guy and Ettema 2011). Therefore, the biological properties of archaea under this category reveal interesting feature of ancient organisms. The representative culturable archaea under this group have now been studied, but little knowledge is available about them except that they are obvious as hyperthermophiles growing optimally at 85 °C (Madigan et al. 2009).

D. Nanoarchaeota

The kingdom Nanoarchaeota has been discovered recently as a group of Archaea and currently having only one representative, *Nanoarchaeum equitans*. *Nanoarchaeum equitans* is a species of very tiny microbe which was discovered in 2002 in a hydrothermal vent off the coast of Iceland. It is a hyperthermophile growing in temperatures near to boiling. Further study showed that *Nanoarchaeum* appears to be an obligatory symbiont on the archaeon genus *Ignicoccus* (Huber et al. 2002). The morphological studies revealed that the cells are only 400 nm diameters in size which made it to place next to the smallest known living organism except possibly nanobacteria and nanomicrobes. Primarily the examination of single-stranded ribosomal RNA (ssrRNA) indicated a considerable difference between this group and the existing well-known kingdoms—Crenarchaeota and Euryarchaeota. On the other hand, the detailed studies related to open reading frames have suggested that the initial sample of ribosomal RNA was biased and *Nanoarchaeum* actually belongs to Euryarchaeota (Brochier 2005). The superphylum DPANN (Diapherotrites, Parvarchaeota, Aenigmarchaeota, Nanoarchaeota, Nanohaloarchaea) was proposed by Rinke et al. (2013) for extremophile archaea.

E. Thaumarchaeota

The recently established phylum of Archaea Thaumarchaeota (derived from the Greek word "*thaumas*" meaning wonder) was proposed in 2008 after whole-genome sequencing was done and has been noticed to have significant difference from other members of hyperthermophilic phylum Crenarchaeota (Brochier-Armanet et al. 2008; Tourna et al. 2011). There are also three known species in addition to *Cenarchaeum symbiosum*: *Nitrososphaera viennensis*, *Nitrososphaera gargensis*, and *Nitrosopumilus maritimus* (Brochier-Armanet et al. 2008). The organisms which closely belong to this phylum are recognized as chemolithoautotrophic ammonia oxidizers and may play significant role in biogeochemical cycles like carbon cycle and nitrogen cycle and other mineralization processes.

This new phylum was proposed in 2008 on the basis of phylogenetic data obtained from the study of the sequences of these organisms such as ribosomal RNA genes and also the presence of a form of type I topoisomerase that was previously thought to be unique to the eukaryotes only (Brochier-Armanet et al. 2008). This research result was later established by further study in 2010 through

Nitrosopumilus maritimus and *Nitrososphaera gargensis*, the genomes analysis of ammonia-oxidizing archaea (AOA), which summarized that these spp. form a distinct relatedness that includes *Cenarchaeum symbiosum*, which was the first member of the new phylum Thaumarchaeota (Spang et al. 2010).

16.4 Ecology of Archaea

Archaea are known to have existed in a broad range of habitats consisting of large part of earth's ecosystem contributing up to 20% of earth's biomass (DeLong 1998; DeLong and Pace 2001). The first discovered archaeon was grouped under extremophiles (Valentine 2007). Interestingly, members of some archaea survive in high temperatures more commonly above 100 °C, as found in extreme conditions like industrial furnace, processing plants, geysers, black smokers, and oil plants. In addition to these, other common habitats include very cold habitats such as cold deserts like Arctic, Antarctica, etc. and highly saline, acidic, or alkaline sites. Whereas, some members of archaea include mesophiles which can grow in moderate conditions like in marshy land, sewage water, the oceans, sea, and soils. Extremophile archaea are members of four major physiologicalgroups. These are the halophiles, thermophiles, alkaliphiles, and acidophiles (Pikuta et al. 2007). These groups are neither strictly restricted to specific groups only nor comprehensive or phylum-specific as some archaea belong to several groups. Despite this, they are very useful from classification point of view.

In the hypersaline habitats, archaea like halophiles which include the genus *Halobacterium* are known to live in extremely saline environments such as salt lakes and crystallizer ponds and have higher microbial population as compared to bacteria at salinities higher than 20–25% (Valentine 2007). Similarly, thermophiles grow best at temperatures above 45 °C in places such as hot springs; the members of hyperthermophilic archaea grow optimum at temperatures greater than 80 °C. Recently a specific archaeal member *Methanopyrus kandleri* strain 116 is noticed to grow at 122 °C, which is the highest registered temperature for any organism (Takai et al. 2008).

The other archaeal group exists in very acidic or alkaline conditions are acidophiles or alkaliphiles (Pikuta et al. 2007). For example, one of the most extreme archaeon acidophiles is *Picrophilus torridus*, which grows at pH = 0 and is equivalent to thriving in 1.2 M sulfuric acid (Ciaramella et al. 2005).

These properties of archaea as resistance to extreme environments have made the possibility to have extraterrestrial life (Javaux 2006). Some of these extremophile habitats are similar to those found on Mars (Nealson 1999) supporting strongly those viable microbes could have been transferred onto planets through meteorites (Davies 1996).

Despite the extreme habitats of archaea, there are several studies which have shown that archaea exist in moderate conditions like mesophilic and thermophilic environments. They are present there sometimes in high numbers at low

temperatures. For example, archaea are common in cold oceanic environments such as polar seas which are known as psychrophiles (Lopez-Garcia et al. 2001). Large archaeal groups of archaea have been reported worldwide in oceans under normal habitats of plankton community of picoplankton (Karner et al. 2001). Although, these archaea may be present in extraordinarily high in numbers (approximately 40% of the microbial biomass), no single species have been isolated and studied in culturable form since they are unculturable (Giovannoni and Stingl 2005).

Similarly, our understanding of archaea as their role in ocean ecology is very limited, so their importance and role on global biogeochemical cycles remain largely unexplored (DeLong and Karl 2005). Some members of marine Crenarchaeota have potential of nitrification, thus affecting nitrogen cycle in oceans (Konneke et al. 2005). However, they may also use other energy sources (Agogue et al. 2008). Significant numbers of archaea are also observed in seafloor sediments, 1 m below ocean bottom, and resistant to extreme pressure known as piezophiles (Teske and Sorensen 2008 and Lipp et al. 2008).

16.5 Biotechnological and Industrial Applications

Extremophilic archaea, especially under thermophiles, acidophiles, or alkaliphiles, are important source of enzymes, proteins, and various metabolites that function under these extreme conditions (Breithaupt 2001; Egorova and Antranikian 2005). The enzymes from psychrophilic archaea and other psychrophiles generally are cold active and heat sensitive, which have major significance in biotechnological applications with particular activity and works at ambient temperature (Vester et al. 2015). Extremozymes from the halophiles have a great economic potential in many industrial processes, including agricultural, chemical, and pharmaceutical applications. These enzymes have multiple uses in human life as well as in the industry. Important enzymes such as DNA polymerases have been obtained from *Thermococcus littoral*, *Pyrococcus woesei*, and *P. furiosus* for their application in polymerase chain reaction (PCR) which has significant role in molecular biology (Satyanarayana et al. 2005). In the same way, Kim and Dordick (1997) reported that an extracellular protease produced by *Halobacterium halobium* has been employed for effective peptide synthesis in water/N0-N0-dimethylformamide.

Recently, a p-nitrophenylphosphate phosphatase (p-NPPase) from *Halobacterium salinarum* was used in an organic medium at very low salt concentrations after entrapping the enzyme in reversed micelles (Marhuenda-Egea et al. 2002). The archaeon *Pyrococcus furiosus* produces thermostable DNA polymerases like Pfu DNA polymerase which has transformed molecular biology through polymerase chain reaction technique, which is a simple and rapid method for DNA cloning.

In the industries, the enzymes like amylases, galactosidases, and pullulanases in some other species of *Pyrococcus* function at over 100 °C allow the food processing

at very high temperatures, such as the production of low-lactose milk and whey (Synowiecki et al. 2006).

The thermophilic archaea produce many enzymes and proteins that have been recorded to thermostable in organic solvents; hence, it may be very useful in green chemistry as eco-friendly processes synthesizing organic compounds (Egorova and Antranikian 2005). Therefore, this stability makes them easier to be use in the structural biology. Also, the counterparts of prokaryotic (bacteria) or eukaryotic enzymes from extremophile archaea are frequently used in structural studies (Jenney and Adams 2008).

On contrary, the wide applications of archaea enzymes and the use of the archaea as organisms in biotechnology are not well developed. A very significant role of methanogenic archaea is in sewage treatment plant, as they are major part of the community of microorganisms that carry out anaerobic digestion of biomass and produce biogas (Schiraldi et al. 2002). In biomining or mineral processing, the acidophilic archaea showed great potential for the extraction of metals from ores including gold, cobalt, and copper (Norris et al. 2000). Most of the members of Halobacteriaceae like *Halobacterium* spp., *Haloferax mediterranei*, and *Haloferax volcanii* are known for producing extracellular protease, poly(β-hydroxybutyric acid) (PHB), bacteriorhodopsin, exopolysaccharides (EPS), etc.

One of the most important features of archaea is that they are the major host of new class of potentially useful antibiotics known as archaeocins. Many archaea have been reported to produce antimicrobials known as archaeocins, i.e., halocins and sulfolobicins, inhibiting closely related species (Aravalli et al. 1998; Prangishvili et al. 2000). A few of these archaeocins have been characterized, but many are believed to be unexplored especially within genus *Sulfolobus* (O'Connor and Shand 2002). These antibiotic compounds differ in structure from bacterial antibiotics so they may have novel modes of action, thus can be used in bacterial disease management. In addition to this, archaeal studies may allow the creation of new selectable markers for their use in archaeal molecular biology (Shand and Leyva 2008).

16.6 Methods and Approaches in Archaea Conservation

A diverse number of microbial strains and species existed, but only 1–10% were characterized, preserved, and used for several applications. Among these, very few archaea species are fully characterized, and the taxonomic position of many is newly described. The genetic resources and application in members of kingdom Archaea have not been fully utilized, and preservation methods in archaea are challenging and sophisticated as compared to other microbial conservation methods. Hence, it becomes important to conserve archaea in their natural habitats. Generally, microorganisms are conserved as "in situ," "ex situ," and "in-factory" form.

"In situ" conservation may be highly effective for halophile, acidophile, thermophile, and alkaliphile group of archaebacteria in their natural ecosystem. Whereas

"ex situ" (in laboratory) conservation practices maintain and preserve isolated genetic stocks and strains/species on synthetic media and are detailed characterized by polyphasic methods. On the other hand, in industrial or commercial application, "in-factory" method of conservation for archaea is used for mass utilization of genetic resources, metabolites, and their useful traits.

16.6.1 In Situ Conservation

Archaea thrive in extreme habitats of hot springs, hydrothermal vent, hypersaline niches, cold deserts like Antarctica and Arctic, stratosphere, extreme pressure and radiations, etc. Likewise, in situ methods of conservation of archaea in their natural habitats are very important since most of the archaea are extremophiles in those particular habitats that are known for their unique characteristics and specific traits with several industrial, biotechnological, and environmental applications. Any disturbance in their natural habitat by physical, chemical, biological, and/or environmental factors will lead to loss of community abundance, genetic diversity, and any particular trait(s). Hence, it is very important to conserve archaeal microflora in their natural habitats. In absence of their specific habitats, certain group of archaea may lose those trait(s) permanently. The Archaea members are widely distributed under extremophilic environments in particular habitats on earth. Most of the hyperthermophilic archaea are radiation resistant (e.g., *Thermococcus gammatolerans* in deep-sea hydrothermal vent). The hyperthermophilic member *Methanopyrus kandleri* strain 116 can grow at 122 °C, whereas *Picrophilus torridus* is reported as extreme acidophilic microbe known to grow at a pH = 0.06. Haloarchaea are extremely halophilic aerobic with pink- to red-pigmented colonies (e.g., *Halobacterium*, *Haloarcula*, *Halorubrum*, *Haloferax*, etc.). These haloarchaea consist of bacterioruberins, carotenoids (C_{50}), bacteriorhodopsin, extracellular hydrolytic enzymes, polyhydroxyalkanoates (PHAs), etc. which have industrial application. They are found in hypersaline habitats like the Dead Sea, salt-soda lakes, salterns, subterranean-solar salts, salted foods, and coastal marshy areas (Grant et al. 2001). The haloarchaea from coastal marshy sediments can grow at lower salinities (Purdy et al. 2004). In Slovenia salterns, two groups of *Halorubrum* were highly dominated in the crystallizers. Burns et al. (2004) observed abundant square haloarchaea from brine samples collected from a crystallizer saltern pond in Geelong, Victoria, Australia.

Two methanogenic archaea have been isolated from permanently frozen Lake Fryxell, Antarctica. Out of the two clusters of methanogens detected, one was predicted to be methanotrophic.

Euryarchaeota was found in the anoxic water level above the sediment, whereas another crenarchaeote was detected just below the oxycline. They may have major role in native biogeochemical cycle, nitrification, and sulfur cycling (Howes and Smith 1990; Karr et al. 2006; Pouliot et al. 2009). Singh et al. (2005) isolated methylotrophic methanogens, *Methanococcoides alaskense* sp. nov. and

Methanosarcina baltica, from anoxic marine sediments in Skan Bay, Alaska. In polar region like an arctic ecosystem of riverine and coastal area, Euryarchaeota community was commonly associated with specific particle-rich waters; however, Crenarchaeota members are particularly reported as free-living natives of marine waters (Galand et al. 2008). Ammonia-oxidizing archaea under Crenarchaeota group in deep sea, which possibly are chemoautotrophs, have been noticed in samples at the depth of 2000–3000 m and ocean sediments (Francis et al. 2005; Nakagawa et al. 2007). Likewise, ammonia-oxidizing archaea were reported in high-altitude soils (4000–6500 m), such as Mount Everest (Zhang et al. 2009). The Antarctic soils are less dominant in archaeal community: mostly belong to Crenarchaeota (Aislabie and Bowman 2010). The polyextremophile archaea, *Sulfolobus acidocaldarius*, thrive at pH = 3 and temperature of 80 °C, which were isolated from Congress Pool, Norris Geyser Basin, Yellowstone National Park, USA. A nitrate-reducing chemoautolithotroph, *Pyrolobus fumarii* (Crenarchaeota), can grow as high as 113 °C (Blochl et al. 1997). In Japanese soils permeated with solfataric gases, the isolated extreme acidophile aerobic heterotrophs, i.e., *Picrophilus oshimae* and *Picrophilus torridus*, grow at pH 0.7 and 60 °C (Schleper et al. 1995). Similarly, acid mine drainage inhabitant of iron in California, *Ferroplasma acidarmanus* found capable to grow at pH = 0, in the presence of sulfuric acid and high concentration of heavy metals like copper, arsenic, cadmium, and zinc (Edwards et al. 2000). Cold-loving archaea in the world's oceans has significant contribution to the biomass (10^{28} cells) in the hypoxic and/or anoxic condition (Horn et al. 2003). The Archaea may act as an effective model organism for astrobiology. The haloarchaea members under Euryarchaeota are found abundantly in oil-contaminated soils and have role as in "in situ" biodegradation in particular geochemical conditions (Al-Mailem et al. 2010; Bonfa et al. 2011; Wang et al. 2011). Archaea are heterogeneous with diverse physiology. They are heterotrophic on several compounds and in combination with chemiosmosis can utilize substrate level phosphorylation (SLP) to synthesize ATP. The energy is conserved by various means. In anaerobic conditions, energy is conserved by anaerobic photorespiration, fermentation, and anaerobic respiration with nitrate by utilizing bacteriorhodopsin through sodium ion-pumping methyltransferase and proton-pumping hydrogenases (Schäfer et al. 1999; Mayer and Müller 2013). Thus, archaea community has great potential to conserve for long-term preservation in their natural habitats for their particular species or strains through adaptations. It may be targeted at particular species or entire ecosystems (Heywood and Dulloo 2005).

16.6.2 Maintenance of Archaea

The maintenance is the process of preserving a particular condition, techniques, method, or situation that is being preserved. The axenic or pure culture is maintained for all future research and references. The cultures may get contaminated from other microbes or strains. Hence, it becomes necessary to have enough stock of cultures in

storage. The sufficient stock may be prepared and subcultured on specialized media with multiple replicas. It is then incubated in BOD at proper temperature to obtained proper growth. The pure culture plates are sealed with Parafilm and kept in refrigerator at 4 °C for suitable time period as per requirement and specification. The culture replicas should be kept in different preservation storage unit to ensure the safety of cultures under any adverse conditions. The stock archaea cultures may be maintained on specialized agar plates and slants with proper condition considering their taxonomic description. The maintenance of haloarchaea on agar plates and slants has limitation as crystals may be formed in medium with shrinkage. Therefore, the cultures are stored at low temperature for proper maintenance and to avoid the practice of frequent subculturing. Also, it may cause mutation in the strain or genetic instability. Therefore, stored cultures must be monitored regularly with periodic observation and if necessary subcultured them. Some culturable archaea may lose viability when kept at longer period of time especially for aerobic archaea. Thus, media should be changed at specific time interval with cultivation specification. The halophilic archaea require subculturing after 5–6 months when stored at 5 °C; however, some strains may show genetic instability by frequent subculturing at variable temperature and medium composition. Nagrale et al. (2015) stated that haloarchaea isolates were highly prone to low-temperature fluctuations with formation of salt crystals in haloarchaea agar. However, strains of *Haloarcula* spp. and *Halorubrum* spp. can be maintained at 4 °C for 2 months without crystallization of salts in haloarchaea agar (Fig. 16.1). But, strain *Haloarcula quadrata* M4 (2) showed genetic instability when preserved in haloarchaea agar slants at room temperature for short period.

Several media have been recommended for the cultivation of various genera and species of family *Halobacteriaceae* (Larsen 1981; Oren 2001a; Tindall 1992; Das Sarma et al. 1995; Rodriguez-Valera 1995). The website of the Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ, http://www.dmsz.de, http://www.microbiol.unimelb.edu.au/micro/staff/mds/HaloHandbook/index.html), and American Type Culture Collection (http://www.atcc.org) provides detailed information on the growth and cultivation of both halophilic Archaea.

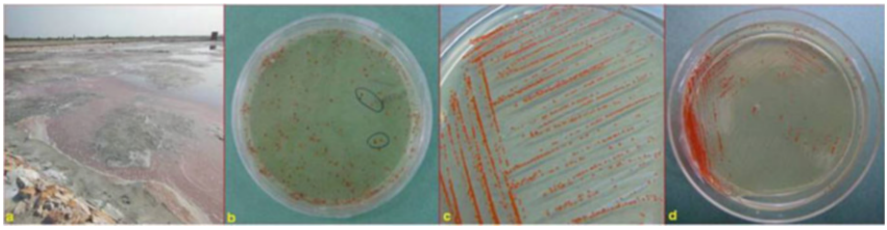

Fig. 16.1 Extremely halophilic archaea: Sampling site, colonies development and strains (**a**) Pink colouration in Sambhar salt lake, Rajasthan (India) by haloarchaea bloom (**b**) Development of pin point colonies on haloarchaea agar at 37 °C after 7 days (**c**) Colonies of strain *Haloarcula marismortui* M3(1) [GenBank accession: KJ526223] (**d**) Colonies of strain *Haloarcula argentinensis* M4(1) [GenBank accession: KJ526221]

Media used for haloarchaea differ significantly both in their salt concentration and ionic composition. The haloalkaliphilic strains are cultivated in medium (pH 9.5) with very low concentrations of divalent cations ($Mg^{2+}$and Ca^{2+}). Some bacteriological peptone (Difco) may cause disintegration of many haloarchaea. However, starch and sugars neutralize toxic effects and enhance growth of some species (Kamekura et al. 1988; Oren 1990). The members of family *Halobacteriaceae* are well suited for growth in dark condition. The haloarchaea media should be amended with antibiotics such as penicillin or ampicillin inhibiting halophilic bacteria. For the isolation and maintenance of halophilic archaea, a higher agar concentration is recommended as high salinity generally interferes with solidification of media.

Haloarchaea cultures may be maintained on agar slants at 4 °C, to be subcultured 3–6 months by specifying their taxa. Loss of character or mutation may occur due to frequent subculturing hence stored by freezing or drying method. Vacuum drying method is quite satisfactory for members of *Halobacteriaceae* and preferred by most of the microbial culture collection centers. Sakane et al. (1992) reported that L-drying has been successfully used for the preservation of certain members of *Halobacteriaceae*, especially aerobic haloarchaea. However, for anaerobic haloarchaea like *Halorhodospira*, *Ectothiorhodospira*, and some other members require special techniques.

Haloarchaea are also stored in liquid nitrogen in specialized media supplemented with DMSO 5% (w/v). It can also be stored at −60 to −80 °C by supplementing media with 10–20% glycerol (Tindall 1992; Hochstein 1988; Jones et al. 1984). *Halanaerobiales* require anaerobic techniques for growth and cultivation. Oren (2001b) suggested boiled anaerobic media amended with nitrogen (80:20) and reducing agents, i.e., cysteine, dithionite, or ascorbate. In addition to this, methanogenic archaea preserved aerobically by freeze and heat drying techniques to store at short and long periods (Bhattad 2012).

16.6.3 Method of Preservation

Different standard techniques are available for the preservation of archaea and other extremophiles. It is also suggested to submit the culturable strains or isolates at recognized culture collection center for publishing research article with proper passport data providing detailed information of the strain or isolate (Table 16.1). Most of the halophilic archaea strains can be preserved in the Petri plates or in the refrigerator at 4 °C for longer periods with suitable sealing. Some strains may lyse rapidly if stored at −20 °C in 20–50% glycerol; however, many strains survive at room temperatures up to 6 months, but this needs to be specified. It is also ported that some members of *Halobacteriaceae* can be preserved well for up to 2 years with cryoprotectants like glycerol and sucrose at −70 °C. The most successful method of haloarchaea storage is in liquid nitrogen storage tank with 15% glycerol mixed in culture media. The cultures can be stored for minimum of 15 years in quality storage tank.

Table 16.1 Archaea cultures available worldwide at different culture collection centers

Sr. no.	Name of the organization	Archaea available	Source
1	Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Inhoffenstraβe 7B 38,124 Braunschweig, Germany	500 strains	https://www.dsmz.de/contact.html
2	American Type Culture Collection (ATCC), 12,301 Parklawn Drive, Rockville, MD-20852-1776, USA, PO Box 1549, Manassas, Virginia, 20,108 1549, USA	14 (Type strains)	https://www.atcc.org
3	Japan Collection of Microorganisms (JCM), RIKEN Bio Resource Center, 3-1-1 Koyadai, Tsukuba, Ibaraki 305-0074 Japan	520 strains	http://jcm.brc.riken.jp/en/
4	National Agriculturally Important Microbial Culture Collection (NAIMCC), ICAR-National Bureau of Agriculturally Important Microorganisms, Kushmaur, Mau Nath Bhanjan-275103 (U.P.), India	17 strains	http://www.mgrportal.org.in
5	Microbial Type Culture Collection and Gene Bank, CSIR—Institute of Microbial Technology Sector 39-A, Chandigarh 160036, India	6 strains	https://www.mtccindia.res.in
6	National Centre for Microbial Resources (NCMR), National Center for Cell Science, First floor, Central Tower, Sai Trinity Building Garware Circle, Sutarwadi, Pashan Pune, Maharashtra 411021, India	16 strains	http://www.nccs.res.in

Connaris et al. (1991) evaluated method for the preservation of the hyperthermophile archaeon *Pyrococcus furiosus.* The application of glass capillary tubes kept over liquid nitrogen with dimethyl sulfoxide (DMSO) is preferred for preservation. Lyophilization techniques result in loss of viability of most of the archaea cultures. *Pyrococcus furiosus* lost viability when preserved by lyophilization. However, this technique was quite successful against hyperthermophile archaea like *Desulfurococcus* and *Thermococcus.* Jannasch et al. (1992) stated that hyperthermophiles like *Pyrococcus* spp. may be isolated from refrigerated as well as oxygenated samples in storage after 5 years.

On the basis of duration, the preservation techniques can be classified as:

- Short-term preservation
- Medium-term preservation
- Long-term preservation

16.6.3.1 Short-Term Preservation

Refrigeration

This is preferred method of storage of haloarchaea at 4 °C for only short period. The cultures can be maintained on haloarchaea agar slants or in Petri dishes for routine study. Petri dishes should be sealed properly with Parafilm to avoid any contamination and maintenance of moisture in agar media. For aerobic archaea, cotton plugs are preferred over screw-capped tubes in sterilized slants with agar media and then

inoculate the culture in aseptic condition. The cultures may be transferred in new agar media with proper time period to maintain viability. Erauso et al. (1993) demonstrated that *Pyrococcus abyssi* sp. nov., a hyperthermophilic archaeon culture, can be stored at 4 °C when gas phase flushed with N_2 to remove H_2S emitted during cultivation. The cultures may be stored for a minimum of 1 year. Arab et al. (2000) stored two novel hyperthermophilic archaea (*Thermococcus aegaeicus* sp. nov. and *Staphylothermus hellenicus* sp. nov.) at 4 °C for a short period. Godfroy et al. (1996) stated that purified culture of *Thermococcus fumicolan*, a novel hyperthermophilic archaeon, was stored at 4 °C for short period of 1 year.

16.6.3.2 Medium-Term Preservation

L-Drying

L-drying or liquid state drying is a method where culture is protected from freezing. The method of drying is practiced in vacuum below 4 °C. Sakane et al. (1992) successfully used L-drying method for long-term preservation of extremely haloarchaea and thermoacidophilic archaea, labile to freeze and freeze-drying. Accelerated storage test is thus effective for estimating the stability of the dried specimens of archaea during preservation. Even the most sensitive archaea, *Thermoplasma*, survived for more than 15 years at 5 °C. Preservation of haloarchaea cultures requires skim milk "sponges" or "plugs" followed by freeze-drier.

Storing of Cultures (Dyall-Smith 2009)

1. Cells are harvested from 20 ml fresh grown culture and resuspended in a prepared solution (per 100 ml):

   Monosodium glutamate: 10 g
   Adonitol (adonite): 1.5 g
   D-Sorbitol: 2.0 g
   Sodium thioglycolate: 0.05 g
   Sodium chloride: 20 g
   Prepare the suspension in 0.1 M phosphate buffer and sterilized by filtration (0.45 μm). Maintain the pH at 7.0

2. Place 1–2 drops of resuspended culture onto a skim milk "sponge," and put in a freeze dryer for drying for half to 1 h.
3. Then, small tube should be kept in larger test tube and sealed by heating.

Opening and Revival Cultures

1. The outer test tube is opened by heating the top over burner.
2. Remove the inner glass tube, and add fresh small quantity of sterilized growth medium onto skim milk sponge or plug, and mix by a Pasteur pipette.
3. Then transfer the contents in sterilized flask with growth medium, and incubate at 37 °C in shaking incubator, and observe the growth.

16.6.3.3 Long-Term Preservation

Ultralow Freezing

Archaea cultures can be stored for several years by ultralow freezing. Ultralow temperature minimizes chemical reaction within culture. The American Type Culture Collection (ATCC) utilizes freeze-drying method for preservation of archaea including several other microorganisms. Higher methanogenic activity has been reported in freeze-dried cultures than heat-dried cultures. In limited oxygen level, higher methanogenic activity of archaea is noticed than in complete anaerobic conditions. In freeze-drying, Bhattad (2012) reported glucose as cryoprotectant, more effective for methanogenic activity compared to heat drying. The *Halohandbook* (Dyall-Smith 2009) demonstrated that haloarchaea strains can be preserved at −80 °C with 80% glycerol and 6% SW solution. Rieger et al. (1997) stated that cryofixation of hyperthermophilic archaea with very high cell densities in cellulose capillary tubes results in improved preservation of their fine structures, whereas Rengpipat et al. (1988) stated that halophilic archaea may be preserved through lyophilization or at −80 °C in anaerobic suspensions with specified media at proper salt concentration and 20% glycerol. Erauso et al. (1993) also stated that pure culture of *Pyrococcus abyssi*, a novel species of hyperthermophilic archaeon, was stored at −80 °C in anaerobic condition with growth medium supplemented with 20% (w/v) glycerol. Huber et al. (2000) demonstrated that two novel hyperthermophilic and chemolithoautotrophic *Ignicoccus* spp. stock cultures can be stored at −140 °C with 5% (v/v) DMSO over liquid nitrogen, and cultures were found viable for a minimum of 3 years. Arab et al. (2000) reported two novel species of hyperthermophilic archaea (*Thermococcus aegaeicus* and *Staphylothermus hellenicus*) which can be stored for long term in pure form at −70 °C in anaerobic condition when supplemented with cryoprotectant 5% (w/v) DMSO (Sigma). Birrien et al. (2011) stated that *Pyrococcus yayanosii*, a novel obligate hyperthermophilic piezophile archaeon, can be kept for long-term storage under anaerobic condition in 1.8 ml cryotubes at −80 °C containing cryoprotectant as DMSO 5% (v/v) (Sigma).

16.7 Conclusion and Perspectives

Several groups of archaea develop in diverse ecosystem and environments. The general classification of archaea is based on rRNA gene sequences to reveal molecular phylogenetics. Most of the culturable archaea members have two main phyla, i.e., Euryarchaeota and Crenarchaeota. The phylum Nanoarchaeota has only one member as *Nanoarchaeum equitans*. The members in other phylum Korarchaeota have generally thermophilic species, sharing characteristics of major phylum, but are close to Crenarchaeota. The culturable halophilic archaea are generally red-pigmented species that belong to Halobacteriaceae which are aerobic or

facultative anaerobe. In 2006, new some smallest-sized group of archaea was detected, designated as archaeal Richmond Mine acidophilic nanoorganisms (ARMAN) consisting of Micrarchaeota and Parvarchaeota. Similarly, a superphylum TACK has been proposed including the members from major phyla. Archaea have significant role in biotechnological and biogeochemical transformation with several applications in industry, pharmaceutical, biotechnology, food, chemical industries, environmental sciences, bioremediation, and ecosystem management. Archaea preservation requires very specific preservation techniques, since they are highly specific in their cultivation parameters and, thus, need more focused research for maintenance, preservation, conservation, and cultivation. Hence, it necessitates for the development of reliable, simple, and durable preservation technique for certain groups of archaea for long-term preservation with stable viability over the years. For effective in situ conservation, the particular habitats and niche must be protected from disturbance by means of any anthropogenic and environmental factors through biodiversity regulatory agency. The specific biomolecules, secondary metabolites, genes, and biopolymers produced by archaea may play important role in industrial growth. Many species of archaea are being utilized as biological model to study extraterrestrial life. The unique feature of domain Archaea exhibits characteristic features from the other domains that continue to stimulate discussions among evolutionary biologists. Thus, culture collections or microbial repository has major role and challenges to preserve this treasure archaeal group by maintaining specific traits for utilization in research areas and industrial application. Likewise, most of the members of domain Archaea are very sensitive for isolation and maintenance in laboratories; hence, there are challenges before scientists and researchers develop modified, novel, and simple technique(s) which will be effective for long-term preservation with distinct characteristics and their maintenance for future use.

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